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TRYPSIN INHIBITORS OF HUMAN SERUM. I. STANDARDIZATION, MECHANISM OF REACTION, AND NORMAL VALUES [1]

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Camus and Gley (1) appear to have been the first to observe that human serum inhibits trypsin, and Ascoli and Bezzola (2) and Brieger and Trebing (3), the first to report that this trypsin inhibiting activity may be increased in some diseases. Since that time the inhibition of trypsin by the serum and plasma of normal and diseased persons has been the subject of several investigations. Grob (4) and Jacobsson (5) have reviewed the earlier literature.

A variety of substrates and trypsin preparations have been used in measuring the inhibition of trypsin by serum and in studying the mechanism of this reaction. The determination of inhibitor levels has received the most attention and results have often been expressed in arbitrary units or referred to the amount of inhibition produced by normal serum. Concentrations in normal human serum have been expressed in terms of micrograms of crystalline trypsin inhibited per ml. of serum by Christensen (6), Peacock and Sheehy (7), Shulman (8), and Jacobsson (5). The average values were 655, about 1,400, 2,200, and 752, respectively. In each case the assay method involved preincubation of trypsin and serum in an alkaline medium. Christensen and Jacobsson, following the suggestion of Kunitz (9), standardized their trypsin preparations by titration with crystalline soybean inhibitor.

Hussey and Northrop (10) and Grob (4), using crude trypsin preparations, found the inhibition by human plasma to be reversible. On the other hand, Shulman (11) and McCann and Laskowski (12) using crystalline trypsin preparations, the former with human serum and the latter with rat plasma, found the inhibition to be stoichiometric and irreversible. It had been recognized that there are two components in human plasma which inhibit trypsin, and Jacobsson (13) showed that these could be demonstrated by zone electrophoresis to be components of the α_1- and the α_2-globulin. He subsequently found (5), using the Anson hemoglobin assay for trypsin, that both inhibitors react reversibly with trypsin, and that the dissociation constant for the inhibitor in the α_1-globulin was 6×10^{-10} and that for the inhibitor in the α_2-globulin, 3×10^{-10}. Having found that urea makes the otherwise irreversible inhibition of trypsin by soybean inhibitor appear to be reversible (5, 14), Jacobsson suggested that the reaction with serum trypsin inhibitors might be irreversible in the absence of urea.

The present report is concerned with some modifications of the casein method of Kunitz (9) for the determination of tryptic activity, with the question of the reversibility of the reaction between trypsin and serum inhibitor, and with certain other factors bearing on the standardization of methods for the determination of trypsin inhibitor levels in serum.

METHODS AND MATERIALS

Estimation of the concentration of trypsin and inhibitor. Salt-free trypsin and soybean trypsin inhibitor (Worthington Biochemicals), twice crystallized and five times crystallized, respectively, were used. Stock solutions of trypsin and of soybean inhibitor were prepared in 0.0025 N HCl and the absorbance was determined at 280 mμ in a Beckman Model DU spectrophotometer. The factors 0.585 for trypsin and 1.10 for soybean inhibitor, given by Kunitz (9), were used to convert absorbance to mg. protein per ml. solution. Working solutions of both enzyme and inhibitor were prepared as indicated below.

Substrate. Three Gm. vitamin-free casein (Pfanstiehl) was suspended in 100 ml. of 0.17 M tris (hydroxymethyl) aminomethane brought to pH 7.6 with HCL. The suspension was heated in a boiling water bath for 15 min-

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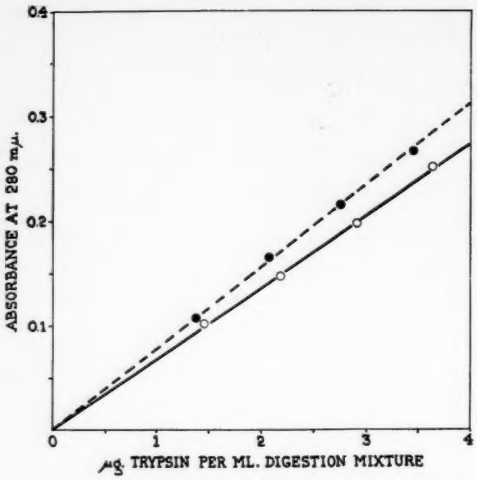

FIG. 1. STANDARDIZATION OF TRYPSIN DIGESTION OF CASEIN

The absorbance of the supernatant from the trichloroacetic acid precipitate of the digest as compared to the trypsin concentration: ○—○, in the absence of added Ca^{++}; ●- - -●, with Ca^{++} added to 4×10^{-3} M in the preincubation mixture before adding substrate.

utes to dissolve the casein. The solution had a final pH of 7.0.

Determination of trypsin activity. The casein digestion procedure of Kunitz (9) was used with some modifications. Since serum inhibitory activity disappears rapidly on exposure to a pH of less than 6, all dilutions were made with 0.17 M tris buffer, pH 7.6, rather than with 0.0025 N HCl as in the original method of Kunitz. A working trypsin solution was prepared by diluting the stock solution 1:10 with the buffer. To serial volumes of 0.1 to 0.8 ml. of the working trypsin solution, sufficient buffer was added to bring the total volume in each tube to 2 ml. These trypsin dilutions and the casein substrate were allowed to incubate separately for 15 minutes at 35° C. in order to attain temperature equilibrium. After this time 1 ml. of 3 per cent casein was added to each of the trypsin dilutions and the mixtures were allowed to incubate at 35° C. Digestion was stopped after 20 minutes by the addition of 6 ml. of 2.5 per cent trichloroacetic acid. Blanks were prepared by adding the trichloroacetic acid to the casein and then mixing with the trypsin dilutions. It was found to be important to allow about one and a half hours after addition of trichloroacetic before removing the precipitate by centrifugation. The absorbance of each supernatant was measured at 280 mμ with a Beckman Model DU spectrophotometer. Absorbance, corrected for the blank value, was plotted against trypsin protein concentration as shown in Figure 1.

The original method of Kunitz employs a 0.5 per cent casein concentration in the final digestion mixture. It was found that the rate of reaction decreased during the 20 minute digestion period, but that a linear rate could be maintained by increasing the final casein concentration to 1 per cent. Under these circumstances the rate also becomes linear with trypsin concentration over a greater range of trypsin concentrations.

Using the higher substrate concentration, some difficulty was encountered with the release of digestion products from the precipitate produced by adding 3 ml. of 5 per cent trichloroacetic acid to the digestion mixture. More satisfactory results were obtained by using 6 ml. of 2.5 per cent trichloroacetic.

These changes, together with the change to preincubation at pH 7.6, have the disadvantage that our specific enzyme activities are not comparable with those obtained by the original method. However, the reliability is improved by employing conditions in which zero order kinetics are obtained. Specific enzyme activity is defined as the increase in absorbance per minute per μg. of trypsin per ml.

Determination of inhibitor activity. A working soybean inhibitor solution was prepared by diluting the stock

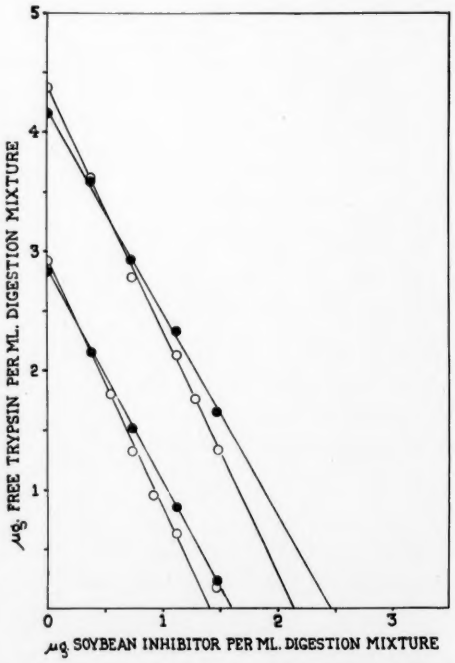

FIG. 2. EFFECT OF CALCIUM ON THE INHIBITION OF TRYPSIN BY SOYBEAN INHIBITOR

Residual trypsin as a function of increasing amounts of soybean inhibitor for two different trypsin concentrations: ○—○, in the absence of added Ca^{++}; ●- - -●, Ca^{++} added to a concentration of 4×10^{-3} M in the preincubation mixture before the addition of substrate.

solution 1:10 with the tris buffer. Serial volumes of this working inhibitor solution were added to a fixed volume of the working trypsin solution and the final volumes adjusted to 2 ml. with tris buffer. Residual trypsin activity was determined by adding 1 ml. of 3 per cent casein and completing the determination of activity described above.

In determining inhibition by serum, the serum was diluted 1:100 or 1:200 with the tris buffer, and serial volumes of these dilutions were added in place of the soybean inhibitor.

Blanks were prepared for the lowest and highest concentration of serum used and intermediate blank values were calculated by interpolation. This correction was not required for soybean inhibitor, which contained no appreciable material absorbing at 280 mμ which was not precipitated by trichloroacetic acid.

Additions of ethylenediaminetetracetate and calcium. Ethylenediaminetetracetate (EDTA) was added as the disodium salt and calcium as $CaCl_2$. The concentrations indicated subsequently are those in the preincubation mixture, before the addition of substrate.

RESULTS

Inhibition of trypsin by soybean inhibitor

The effect of Ca^{++}. Since Ca^{++} has been shown to increase the stability of trypsin at an alkaline pH (15, 16) and to increase the esterase and amidase activity of trypsin (17), it was decided to investigate the effect of this cation on the proteolysis of casein by trypsin and on the observed combining ratio of trypsin and soybean inhibitor. Results are shown in Figure 1 and Figure 2. As indicated in Figure 1, the addition of Ca^{++} to a concentration of 4×10^{-3} M increases the specific activity of the trypsin from 3.4×10^{-3} to 3.9×10^{-3}. The intercept obtained by extrapolating the curves in Figure 2 to zero free trypsin gives the amount of inhibitor which should completely inhibit the total amount of trypsin added. Combining ratios can be calculated from these values. Combining ratios obtained in this manner are CT/I [3] = 2.10 in the absence of Ca^{++}, and CT/I = 1.80 for Ca^{++} added to 4×10^{-3} M. Thus Ca^{++} appears to have increased the activity of this sample of trypsin by about 15 per cent and the observed combining ratio has decreased correspondingly.

Effect of ethylenediaminetetracetate (EDTA). The results of similar experiments showing the effects of Ca^{++}, EDTA and Ca^{++} plus EDTA on the specific activity and on the observed combining ratio with soybean inhibitor of two trypsin preparations are summarized in Table I. The trypsin preparations represent the same original crystalline trypsin but since the experiments were performed two years apart, they are considered here as different samples. Ca^{++} seems to exert a maximum effect at 10^{-3} M. In each case, the decrease in combining ratio parallels the increase in specific activity. Low concentrations of EDTA decrease the specific activity of trypsin in the absence of added Ca^{++}. This may indicate the presence of traces of Ca^{++} in the reagents, or of tightly bound Ca^{++} or other cations in the trypsin molecule. As was observed by Green and Neurath (17), higher concentrations of EDTA have an activating effect. Although EDTA at lower concentrations may cause a 28 per cent decrease in trypsin activity, the combining ratio with soybean inhibitor remains unchanged. EDTA completely counteracts the effect of added Ca^{++}.

Table II summarizes the results of similar experiments performed with serum.[4] The findings

TABLE I

The effect of Ca^{++}, ethylenediaminetetracetic acid and ethylenediaminetetracetic acid plus Ca^{++} on two trypsin samples and on their reaction with soybean inhibitor

Added Ca^{++} M/L.	EDTA* M/L.	Specific enzyme activity ×10³	CT† / I
	Trypsin sample 'a'		
0	0	3.4	2.10
4×10^{-3}	0	3.9	1.80
4×10^{-3}	10^{-2}	2.7	2.16
	Trypsin sample 'b'		
0	0	3.2	2.28
5×10^{-5}	0	3.4	
10^{-3}	0	3.7	
4×10^{-3}	0	3.7	1.88
10^{-2}	0	3.7	
0	10^{-4}	2.9	
0	10^{-3}	2.3	2.29
0	10^{-2}	2.7	
2.25×10^{-3}	2.5×10^{-3}	2.8	2.31

* Ethylenediaminetetracetic acid.
† Micrograms of trypsin inhibited by 1 μg. soybean inhibitor.

[3] CT/I = Micrograms of trypsin inhibited by 1 μg. soybean inhibitor.

[4] The sera used in these experiments were samples of pooled sera obtained from the Chemistry Laboratory of the Los Angeles County General Hospital.

were qualitatively similar to those obtained in experiments with soybean inhibitor.

The specific activities for both Tables I and II were calculated from trypsin concentrations based on the absorbancy of the trypsin solutions. Values of CT/I for Table I and of the amount of trypsin apparently inhibited per ml. of serum (Table II) were calculated on the same basis. The corrected values for the inhibition of trypsin by serum, which are given in the last column of Table II, were calculated on the assumption that soybean inhibitor combines with trypsin on an equimolar basis under the same conditions. This basis for standardization will be considered in more detail in the discussion.

Effect of preincubation time

Since it is known that trypsin undergoes autolysis in alkaline solution and that Ca^{++} increases the stability of trypsin at an alkaline pH (7, 8), our results indicated that Ca^{++} was exerting a protective action during the alkaline preincubation period. This possibility was investigated by determining trypsin activity after varying periods of preincubation. Working trypsin solutions were prepared by dilution of the stock solution with 0.0025 N HCl or 0.0025 N HCl plus 4×10^{-3} M Ca^{++}. 1.8 ml. of the tris buffer or 1.8 ml. of the tris buffer plus 4×10^{-3} M Ca^{++} were allowed to come to 35° C. and 0.2 ml. portions of the working trypsin solution were added. At various intervals 1 ml. of 3 per cent casein was added and proteolytic activity was determined by adding 0.2 ml. of the working trypsin solution to a mixture of buffer and casein. Subsequent activities were calculated as per cent of initial activity. Results are shown in Figure 3. As was expected, trypsin undergoes a loss of activity at pH 7.6. The initial rate of decay is quite rapid but gradually levels off. Ca^{++} does indeed afford partial protection against this loss in activity. After 15 minutes preincubation at 35° C., about 70 per cent of the original activity remains in the absence of added Ca^{++}, while 85 per cent remains when Ca^{++} is added.

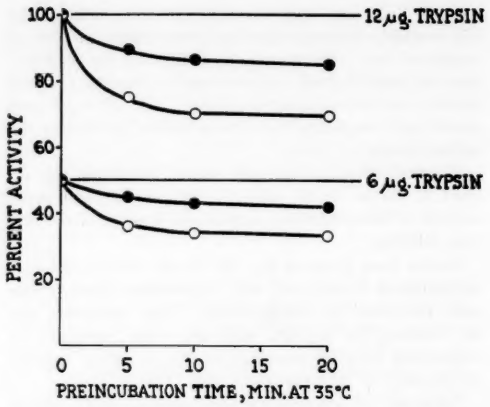

FIG. 3. THE EFFECT OF PREINCUBATION OF pH 7.6 AND 35° C. ON THE ACTIVITY OF TRYPSIN

Expressed as the per cent of initial activity for 12 μg. trypsin: O—O, in the absence of added Ca^{++}; ●---●, in the presence of 4×10^{-3} M Ca^{++} in the preincubation mixture.

TABLE II

The effect of Ca^{++} and ethylenediaminetetracetic acid on trypsin and its inhibition by serum

Added Ca^{++} M/L.	EDTA* M/L.	Specific enzyme activity ×10³	Trypsin inhibited: Apparent mg./ml. serum	Trypsin inhibited: Corrected† mg./ml. serum
Pooled serum sample A				
0	0	2.5	2.38	1.00
4×10^{-3}	0	3.9	1.66	1.11
0	10^{-4}	2.2	2.26	0.95
Pooled serum sample B				
0	0	2.9	2.61	1.24
4×10^{-3}	0	3.9	1.90	1.27
0	10^{-3}	2.1	2.64	1.26

* Ethylenediaminetetracetic acid.
† Corrected on the basis of inhibition by soybean inhibitor under the same conditions (see text).

Dissociation of trypsin-serum inhibitor complex

The following experiments were carried out with preincubation for 15 minutes at pH 7.6, in the presence of 4×10^{-4} M Ca^{++}. Under these conditions, 1 mg. of crystalline soybean inhibitor was found to inhibit 1.8 mg. of our trypsin preparation. Since, as indicated in the discussion, 1 mg. of soybean inhibitor should inhibit 1.2 mg. of trypsin, apparent trypsin concentrations were corrected by a factor of 1.2/1.8.

The effect of increasing amounts of serum on the tryptic proteolysis of casein is shown in Figure 4a. Departure from stoichiometric inhibition with higher concentrations of serum indicates dis-

sociation of a trypsin-inhibitor complex. The same data are plotted in Figure 4b as per cent inhibition by increasing amounts of serum, showing that the inhibition is proportional to the amount of serum added up to about 70 per cent inhibition. This assumes significance in the determination of serum trypsin inhibitor levels, since only values obtained at less than 70 per cent inhibition yield a proportionality between inhibition of activity and the amount of inhibitor present.

Assuming the affinities of the two inhibitors in serum for trypsin are approximately the same, as was found by Jacobsson, that 1 M of each inhibitor combines with 1 M of trypsin, and that the inhibition is noncompetitive,[5] a dissociation constant was calculated from the equation:

$$K_{dis} = \frac{FT\,FI}{CT},$$

where FT and FI represent free trypsin and free inhibitor, respectively, and CT represents combined trypsin. FT was obtained by reference to the activity curve and CT by subtracting FT from the total trypsin added. If the assumptions enumerated above are correct, the ml. of serum obtained by extrapolation of the curves in Figure 1a contain a total number of moles of inhibitor equal to the total moles of trypsin added. FI is then obtained by subtracting CT from the total amount of inhibitor for each serum concentration. All concentrations were changed to moles per liter, using 24,000 as the molecular weight of trypsin (13). The dissociation constant calculated in this manner has the value 8×10^{-10} M. In Figure 5, the molar concentration of combined trypsin is plotted against the molar concentration of inhibitor. The solid line represents the theoretical curve calculated from the dissociation constant 8×10^{-10} M.

[5] This assumption may not be true in the strict sense of the word. However, a threefold decrease in substrate concentration had no effect on the calculated K_{dis}.

Serum trypsin inhibitor levels in normal persons

Serum trypsin inhibitor levels were determined for 40 apparently normal persons between the ages of 20 and 50 years. These were laboratory personnel, medical students, and professional blood donors. The mean for males was found to be 1,025 and for females 1,037; therefore both sexes were considered together. The mean was found to be 1,032 μg. trypsin bound per ml. serum, with a standard deviation of plus or minus 127 and a standard error of the means of plus or minus 6.3 μg. per ml. serum.

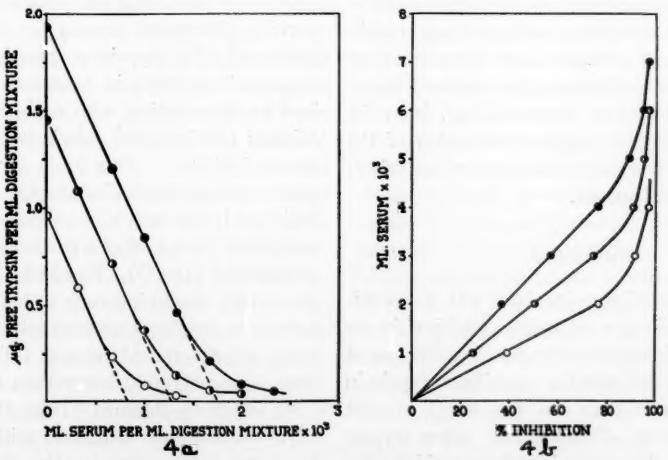

FIG. 4. INHIBITION OF TRYPSIN BY INCREASING CONCENTRATIONS OF HUMAN SERUM

Three trypsin concentrations, plotted in 4a as free trypsin as compared to the quantity of serum; and the same data in 4b as per cent inhibition as a function of the amount of serum added.

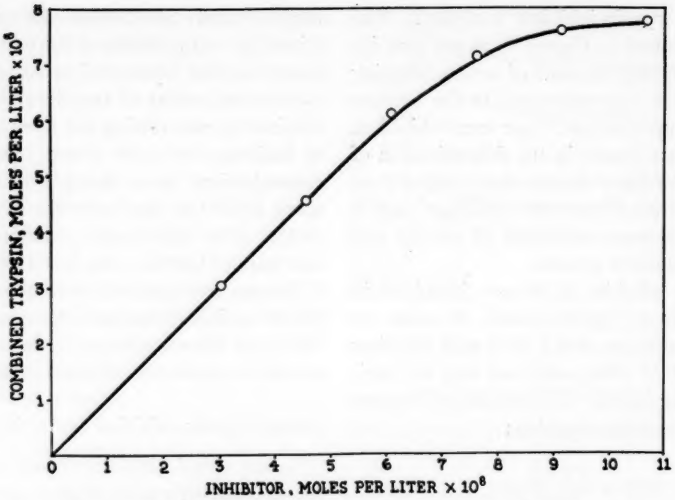

FIG. 5. DISSOCIATION OF TRYPSIN-SERUM INHIBITOR COMPOUND

The points represent experimental values for combined trypsin as a function of increasing total serum inhibitor. The curve is calculated assuming that trypsin and serum inhibitor combine reversibly in equimolar quantities.

Analysis of the differences between duplicate determinations gave a standard deviation for single determination of plus or minus 1.6 per cent and the standard deviation between duplicate analyses on different days was plus or minus 1.0 per cent. At the mean normal value these would be plus or minus 16 and plus or minus 10 μg. trypsin inhibited per ml. of serum. Since determinations were always done in duplicate, the observed standard deviation of plus or minus 127 μg. from the mean normal value is largely a reflection of the variation within the population rather than of errors in the determinations.

DISCUSSION

The instability of trypsin above pH 5 complicates the determination of trypsin inhibitor in serum, since this inhibitor undergoes inactivation at a pH below 6. Although Ca^{++} stabilizes trypsin in alkaline solution, it does not completely prevent the loss of activity. Consequently, when trypsin is preincubated with serum inhibitor at pH 7.6 the trypsin activity decreases as a result of inactivation as well as from combination with inhibitor.

It would be desirable to express inhibitor concentrations in serum in terms of the amount of trypsin inhibited per unit volume of serum. In the present study we have determined trypsin concentrations by measuring the absorbance of the trypsin stock solution at 280 mμ, but such a measure of trypsin concentration is subject to considerable error because of the presence of protein impurities, denatured trypsin, or trypsin autolysis products in the trypsin preparation. Kunitz (9) suggested that trypsin preparations be standardized by the reaction with soybean inhibitor. Jacobsson (5) has done this in his study of trypsin serum inhibitor. This basis for standardization seems well justified, since highly purified soybean inhibitor is commercially available and it has been established that 1 M of trypsin combines with 1 M of inhibitor (18–20). Kunitz had originally taken the weight combining ratio to be 1, but taking the molecular weight of soybean inhibitor to be 20,000 (21), the theoretical value of CT/I would be 1.2. This is considerably lower than the lowest value, 1.80, which we obtained (Table II). If the values of CT/I obtained with and without added Ca^{++} are corrected by assuming that the loss of activity during preincubation at pH 7.6 also represents loss of trypsin able to combine with inhibitor, the corrected values of CT/I become essentially the same in both cases, and average 1.63. This would sug-

gest that some 26 per cent of the absorbance of our trypsin stock solution at 280 mμ was due to inert material.

It might be argued that essentially the same results could be accomplished by standardizing results according to the specific activity of the trypsin. Assuming that both soybean and serum inhibitor combine only with active trypsin, the results obtained with and without added calcium can be harmonized in this way, but this method fails in the experiments with added EDTA. If we take the behavior in the presence of Ca^{++} as a base, trypsin sample 'a' (Table I) has a specific activity of 3.9×10^{-3} and gives a value of 1.80 for CT/I. In the absence of added Ca^{++}, the specific activity is 3.4×10^{-3} and gives a value of 2.10 for CT/I. In the absence of added Ca^{++}, the specific activity is 3.4×10^{-3} or 13 per cent less. Taking this to mean that there is 13 per cent less trypsin than in the presence of Ca^{++}, the observed value of CT/I of 2.10 should be reduced to 1.83, in good agreement with the value of 1.80. However, in the presence of EDTA, the specific activity is reduced by 31 per cent to 2.7. This would lead to a correction of the observed value of CT/I from 2.16 to 1.50. Similar results are obtained with serum inhibitor. The results given in Table II, if corrected for specific activity, would give the same values for mg. trypsin per ml. serum for measurements made with and without added Ca^{++}, but a different value in the presence of EDTA.

The results obtained with serum, measured with or without aded calcium or EDTA, do give the same result if based on the amount of the trypsin available for combination with soybean inhibitor under the same conditions. The corrected values given in the last column of Table II were obtained by assuming that the value of CT/I for soybean inhibitor is 1.2 under all conditions. For example, having an observed value of CT/I of 1.80 for soybean inhibitor in the presence of Ca^{++}, it was assumed that the trypsin concentration was 1.20/1.80 or 0.67 as great as indicated by the spectrophotometric determination on the stock trypsin solution. Consequently, rather than having 1.66 mg. of trypsin inhibited by 1 ml. of serum, only 0.67×1.66, or 1.11 mg. was actually inhibited.

The behavior in EDTA remains unexplained, however. It would be logical to assume that the reduced specific activity in the presence of lower concentrations of EDTA was due to the sequestering of traces of Ca^{++} or other activating or stabilizing ions. However, the effects on observed values of CT/I are not consistent with those obtained by adding Ca^{++}.

The results which have been presented indicate that the inhibition of trypsin by human serum is reversible and that although the dissociation is slight, it should be considered in the determination of serum trypsin inhibitor levels. The value for the dissociation constant is probably correct only within an order of magnitude, since it was calculated from very low residual trypsin activities. The calculation also involves certain assumptions which are probably not strictly true, namely that both inhibitors in serum have the same affinity for trypsin and that the inhibition is noncompetitive. However, the satisfactory agreement between observed and theoretical results indicates that any difference in the affinities of the two inhibitors for trypsin is less than an order of magnitude and that if the inhibition is competitive, it must be of the type postulated by Green (22) for soybean trypsin inhibitor and pancreatic trypsin inhibitor.

The average value of 1,030 μg. trypsin per ml. serum for the inhibitor level in normal, human serum is somewhat higher than those reported by Christensen, 655, and by Jacobsson, 752, and lower than those obtained by Peacock and Sheehy, about 1,400, and by Shulman, 2,200. The latter two estimates failed to take into account the autolysis of trypsin during the alkaline preincubation period, which would contribute to the high values obtained. Christensen standardized his trypsin preparation with soybean inhibitor. However in Christensen's method of assay, the highest dilution of serum which allowed a clot to be formed in the presence of trypsin, thrombin and fibrinogen was assumed to contain a concentration of serum inhibitor equivalent to the amount of trypsin used in the test. Thus his measurements were made in the region of dissociation of the trypsin-inhibitor complex and would be expected to yield low values. Jacobsson also standardized his trypsin with soybean inhibitor using the casein digestion method, but his serum inhibitor determinations were carried out with hemoglobin as the substrate. Using this procedure for soybean inhibitor, he reported an apparent combining ratio of 1.54 Gm. trypsin per Gm. soybean inhibitor, rather

than 1.85 when the casein substrate was used. We believe that the combination with soybean inhibitor should be measured under precisely the same conditions as the serum measurements for which it will serve as a standard. On this basis, and using a theoretical value of 1.2 Gm. trypsin as we have, rather than the value of 1 Gm. trypsin per Gm. soybean inhibitor which was used by Jacobsson, his average value becomes 1,080. This is in good agreement with our value of 1,032.

SUMMARY

The Kunitz casein method for the measurement of trypsin activity has been modified by increasing the substrate concentration and changing the conditions for precipitation of undigested substrate (9). The modifications result in zero order kinetics.

Using this assay procedure, the measurement of trypsin inhibitor activity in serum has been examined with respect to standardization. During the required preincubation of inhibitor and trypsin at pH 7.6, there is a loss of trypsin activity which is initially rapid, but becomes essentially zero after 15 to 20 minutes. The presence of Ca^{++} decreases this rate of decay, but does not afford complete protection.

The decrease in trypsin activity during preincubation produces an equivalent decrease in the amount of trypsin available for combination with both soybean and serum trypsin inhibitors, as judged by the behavior with or without added Ca^{++}. In the presence of EDTA the specific enzymatic activity of the trypsin may be further reduced, but this does not result in a comparable reduction in the amount of trypsin apparently able to combine with either serum or soybean inhibitor.

The results suggest that the soundest basis for standardization of trypsin inhibitor values in serum is the assumption that the amount of trypsin available for binding is the same as that which combines with crystalline soybean inhibitor under the same conditions that are employed for the measurement of the serum inhibitor.

The inhibition of trypsin by human serum has been shown to be reversible, and the significance of this in the determination of serum trypsin inhibitor levels has been pointed out. A dissociation constant of 8×10^{-10} M has been calculated for the trypsin-inhibitor complex. Certain assumptions involved in the calculation have been discussed.

It has been found that 1 ml. of normal serum will inhibit 1.03 ± 0.13 mg. of trypsin, and shown that this can be harmonized with Jacobsson's results. Reasons are presented for the differences from other reported values.

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PRESSURE–VOLUME CHANGES IN THE FOREARM VEINS OF MAN DURING HYPERVENTILATION [1, 2]

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Peripheral venous pressure is known to fall during voluntary hyperventilation (2). Méan (3) found this pressure reduction to be accompanied by a decrease in the volume of small segments of superficial forearm veins. He regarded the volume change as a mechanical phenomenon secondary to the fall in venous pressure and the aspirating effect of the ventilatory efforts. In more recent studies (4, 5) the pressure in temporarily isolated forearm veins was found to rise during overbreathing, indicating an increase in venous tone. From these reports it is evident that the forearm venous volume may decrease during hyperventilation and that the change may be attributed to two factors—a fall in intraluminal pressure and active venous constriction. The experiments reported here were designed to assess the importance of each of these factors in the intact human forearm and to determine if the venous responses to hyperventilation with associated hypocapnia are different from the responses to hyperventilation when carbon dioxide is added to the inspired air.

METHOD AND PROCEDURE

Forearm venous pressure-volume curves were obtained by the plethysmographic method of Litter, Wood, and Wilkins (6, 7). In this procedure the forearm is enclosed in a tall plethysmograph and water is added to a level such that the external pressure on the arm is greater than venous pressure but less than diastolic arterial pressure. The arterial inflow continues and drives venous pressure in the segment of limb within the plethysmograph to a height greater than that of the water column. The difference between the pressure within the veins and the pressure surrounding the veins is the *effective* venous pressure. Under these conditions it is a small positive reproducible value ranging from 0.5 to 1.0 mm. Hg (8). For practical purposes it is regarded as zero pressure. The volume of blood in the veins at this effective pressure is small and constant (9). After the limb is fixed, a pneumatic cuff about the arm proximal to the plethysmograph is inflated by 1.0 mm. Hg increments until the first perceptible increase in limb volume occurs. From this point of "zero" effective pressure an additional pressure of 30.0 mm. Hg is applied to the arm in 5.0 mm. increments. This raises *effective* pressure in the veins of the forearm segment within the plethysmograph from 0 to 30 mm. Hg by the same increments of 5.0 mm. Hg. The increase in forearm venous volume caused by each pressure increment is recorded. The pressure-volume curve is constructed by plotting each volume level achieved at inflow-outflow equilibrium, expressed in ml. per 100 ml. of forearm tissue, against the corresponding level of effective venous pressure (Figure 1). The curve so obtained is convex toward the volume axis. If the veins become less distensible (Figure 1), a subsequent curve will fall below the control curve, nearer the pressure axis. Conversely, if the veins become more distensible, the subsequent curve falls above the control curve, nearer the volume axis. The final point on the curve is the venous volume which exists at an effective venous pressure of 30 mm. Hg. This volume value in a sense represents the venous distensibility since a high value would be associated with a curve of increased slope and a low value with a curve of decreased slope. For the sake of simplicity the venous volume at an effective pressure of 30 mm. Hg is termed arbitrarily the *venous distensibility.* A high value indicates relative venous dilatation and a low value relative venous constriction.

The pressure-volume curve obtained in this fashion expresses the volume to which the forearm venous system is distended by any level of effective venous pressure between 0 and 30 mm. Hg. Assuming that the venous pressure-volume characteristics are the same in both arms, the naturally occurring venous volume of the forearm segment is the volume coordinate of that point on the curve which corresponds to the natural venous pressure (equals effective venous pressure) in the opposite forearm. If a stimulus such as hyperventilation produces venous constriction and also a fall in venous pressure, the decrease

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in volume caused by each of these factors alone may be determined. The volume coordinate of the point on the hyperventilation curve which corresponds to the resting venous pressure is the venous volume which would have existed had the total volume reduction been caused by venous constriction alone (Figure 1). The difference between this value and the venous volume which actually existed during the stimulus represents the reduction in venous volume caused by the fall in pressure alone.

Twenty experiments were performed on 16 healthy men 21 to 34 years of age. Room temperature was maintained at 78° to 80° F. and plethysmographic water temperature at 89° F. Venous pressure was measured in the dependent left antecubital vein and venous distensibility in the right forearm as previously described (10) with minor execptions. Forearm volume changes were measured by means of two partially immersed electrodes [4] (11) which sensed changes in the height of the water in a vertical cylinder attached to the top of the plethysmograph. The diameter of the cylinder was such that maximal volume increases within the plethysmograph raised the water level only a few millimeters. This increase in the height of the water was not great enough to produce a measurable error in effective venous pressure. The tandem plethysmograph previously described (8) was not used. Instead a plethysmograph was employed which contained a very long segment of forearm. With such an instrument volume changes in the incompletely pressurized cone of tissue near the proximal end of the plethysmograph represent only negligible fractions of the total volume change within the apparatus unless the diameter of the forearm is unusually large.

During control periods venous distensibility and pressure were measured intermittently. In 11 experiments end-expiratory CO_2 concentration was monitored with a Liston-Becker CO_2 analyzer. Venous pressure was measured with a Statham 0 to 5 cm. Hg pressure transducer. Volume, pressure and CO_2 concentration were recorded simultaneously using a Sanborn direct-writing oscillograph. Respiratory minute-volume was measured using the gas meter method (12).

After values for venous distensibility and pressure became stable the subject was asked to hyperventilate. In the first 9 experiments the subject was instructed to overbreathe to the point of symptoms by means of maximal inspiratory efforts with passive expirations. In the final 11 experiments the subjects overbreathed in the same way with prompting in an effort to maintain a reduction in end-expiratory CO_2 tension of about 15 mm. Hg. The baseline venous volume of the forearm was recorded continuously during the initial portion of the hyperventilation period and no significant changes were observed. After two to three minutes of hyperventilation or after stabilization of respiratory rate and depth or end-expiratory CO_2 tension, one or two measurements of venous distensibility were made. The overbreathing was then stopped and observations were continued until control values returned. In the final 11 experiments hyperventilation was repeated with 5 or 7 per cent CO_2 and 21 per cent oxygen in the inspired gas. An effort was made to reproduce the same quality and volume of overbreathing which prevailed during the first part of the experiment. After one or two observations on venous distensibility and pressure the hyperventilation was stopped and the measurements were continued until control values returned.

After the experiment venous pressure-volume curves were constructed. The venous volume which existed during each measurement of distensibility was obtained by drawing a line perpendicular to the pressure axis from the natural venous pressure value (Figure 1). The venous volume which would have existed during hyperventilation if venous pressure had not changed was determined by applying the resting venous pressure value to the hyperventilation curve (Figure 1). Resting values for venous distensibility, volume and pressure represent the final set of resting measurements. Values reported during hyperventilation represent the first set of

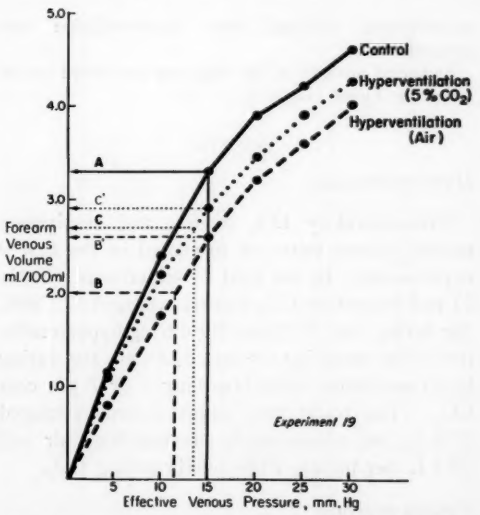

FIG. 1. VENOUS PRESSURE-VOLUME CURVES OBTAINED DURING A CONTROL PERIOD, DURING HYPERVENTILATION WHILE BREATHING AIR AND DURING HYPERVENTILATION WHILE BREATHING 5 PER CENT CO_2

A is the forearm venous volume during the control period. B is the forearm venous volume during hyperventilation while breathing air. B' is the forearm venous volume which would have existed during hyperventilation while breathing air if venous pressure had remained unchanged. C is the forearm venous volume during hyperventilation while breathing 5 per cent CO_2. C' is the forearm venous volume which would have existed during hyperventilation while breathing 5 per cent CO_2, if venous pressure had remained unchanged.

[4] Constructed by Sanborn Company, Waltham, Massachusetts.

TABLE I
Hyperventilation

Experiment No.	End-expiratory CO_2 tension			Ventilation		
	At rest	During hyperventilation		At rest	During hyperventilation	
	Breathing air		Breathing 5 or 7% CO_2*	Breathing air		Breathing 5 or 7% CO_2*
	mm. Hg	*mm. Hg*	*mm. Hg*	*L./min.*	*L./min.*	*L./min.*
10	37.8	27.4	62.2		24.4	38.4
11	42.3	25.6	58.7		28.4	48.1
12	46.0	22.4	65.2		43.5	65.9
13	46.9	25.4	57.0	7.8	28.8	47.5
14	34.3	22.3	53.0		27.0	38.4
15	34.9	21.7	39.9	5.1	45.2	38.0
16	42.1	22.6	45.8		25.0	37.1
17	45.8	26.8	52.6		23.5	21.5
18	48.3	29.3	52.2	5.8	16.3	19.9
19	48.2	25.2	55.2		22.6	30.5
20	43.1	25.8	49.8	6.5	21.2	26.0
Average	42.7	25.0	53.8	6.3	27.8	37.4
Standard Deviation	5.14	2.44	7.17	1.06	8.90	13.30

* Seven per cent CO_2 was used in Experiments 10 to 14; 5 per cent CO_2 was used in Experiments 15 to 20.

measurements obtained after hyperventilation was initiated.

Statistical analysis of the data was performed as described by Fisher (13).

RESULTS

Hyperventilation

End-expiratory CO_2 tension and respiratory minute-volume were not measured in the first 9 experiments. In the final 11 experiment (Table I) end-expiratory CO_2 tension averaged 42.7 mm. Hg during rest, 25.0 mm. Hg during hyperventilation while breathing air, and 53.8 mm. Hg during hyperventilation while breathing 5 or 7 per cent CO_2. The respiratory minute-volume averaged 27.8 L. per minute while overbreathing air and 37.4 L. per minute while overbreathing CO_2.

Venous pressure

Dependent forearm venous pressure fell in 17, increased in 2 and remained unchanged in 1 of 20 experiments during hyperventilation while breathing air (Table II). The average fall in pressure of 1.73 mm. Hg was significant ($p < 0.001$).

Venous pressure increased in 6, decreased in 3 and remained unchanged in 2 of the 11 experiments in which hyperventilation was repeated with 5 or 7 per cent CO_2 in the inspired gas. The average change in pressure was an *increase* of 0.77 mm. Hg. In the same 11 experiments venous pressure decreased in 10 and remained unchanged in 1 during hyperventilation while breathing air; the average change in pressure was a *decrease* of 2.24 mm. Hg. This venous pressure response observed while overbreathing air was significantly different from that observed while overbreathing CO_2 ($p < 0.001$).

Venous distensibility

Active venous constriction occurred in 19 of 20 experiments (Table II) during hyperventilation while breathing air. This reduction in venous distensibility was significant ($p < 0.001$) and averaged 0.87 ml. per 100 ml. of forearm tissue.

Active venous constriction occurred in each of the final 11 experiments during hyperventilation while breathing air and the decrease in venous distensibility averaged 0.60 ml. per 100 ml. Active venous constriction also occurred in each of these 11 experiments when hyperventilation was repeated while breathing 5 or 7 per cent CO_2 and the reduction in venous distensibility averaged 0.52 ml. per 100 ml. The venoconstrictor response observed while overbreathing air was not significantly different from that observed while overbreathing CO_2 ($p > 0.4$).

Venous volume

In 20 experiments the resting forearm venous volume averaged 2.88 ml. per 100 ml. of forearm

TABLE II

Forearm venous responses during hyperventilation

Experiment No.	Venous pressure			Venous distensibility			Venous volume				
	At rest	During hyperventilation		At rest	During hyperventilation		At rest	During hyperventilation			
	Breathing air	Breathing air	Breathing 5 or 7% CO_2	Breathing air	Breathing air	Breathing 5 or 7% CO_2	Breathing air	Breathing air		Breathing 5 or 7% CO_2	
	mm. Hg	*mm. Hg*	*mm. Hg*	*ml./100 ml.*	*ml./100 ml.*	*ml./100 ml.*	*ml./100 ml.*	*ml./100 ml.*	*ml./100 ml.**	*ml./100 ml.*	*ml./100 ml.†*
1	17.1	18.6		3.9	2.3		3.2	1.9	1.8		
2	9.5	9.1		5.4	3.9		3.1	1.9	2.1		
3	17.9	15.9		4.1	3.5		3.4	2.8	3.0		
4	16.0	13.0		4.3	3.8		3.3	2.7	3.0		
5	12.3	13.5		3.9	2.8		2.2	1.2	1.1		
6	15.7	10.4		4.9	4.9		3.4	2.9	3.2		
7	15.8	15.1		4.6	1.9		3.6	1.2	1.3		
8	17.2	17.0		4.1	2.5		3.2	1.7	1.7		
9	12.5	11.5		4.4	3.3		3.0	2.5	2.9		
10	13.7	11.0	15.5	3.1	2.2	2.4	2.1	1.2	1.3	1.6	1.5
11	14.0	11.3	17.5	3.8	3.6	3.3	2.6	2.3	2.6	2.3	2.0
12	8.8	5.0	8.5	3.5	2.6	3.0	1.5	0.4	0.8	1.2	1.3
13	10.0	6.3	12.0	4.9	4.7	4.3	3.1	2.2	3.1	2.8	2.5
14	12.5	11.3	15.0	4.1	3.9	3.3	2.7	2.2	2.3	2.0	2.2
15	9.0	9.0	9.0	3.5	2.4	2.5	1.9	1.0	1.0	1.2	1.2
16	8.5	7.5	9.0	3.5	2.6	3.1	1.4	0.9	1.0	1.1	1.0
17	12.5	11.0	12.5	5.7	5.3	5.5	3.8	3.5	3.6	3.5	3.5
18	15.0	13.0	15.5	3.0	2.8	2.9	2.3	1.7	1.9	2.2	2.2
19	15.0	11.5	13.5	4.6	4.0	4.3	3.3	2.0	2.6	2.7	2.9
20	14.0	11.5	13.5	6.4	5.4	5.8	4.4	3.5	3.7	3.8	3.9
Expers. 1–20											
Average	13.35	11.63		4.29	3.42		2.88	1.99	2.20		
S. D.‡	2.965	3.405		0.865	1.128		0.956	0.889	0.926		
Expers. 10–20											
Average	12.09	9.85	12.86	4.19	3.59	3.67	2.65	1.90	2.17	2.22	2.20
S. D.	2.545	2.541	3.033	1.097	1.167	1.153	0.942	1.003	1.045	0.921	0.946
Expers. 1–20											
Aver. dif.§		−1.73			−0.87			−0.89	−0.68		
S. E.‖		0.391			0.148			0.110	0.130		
p¶		<0.001			<0.001			<0.001	<0.001		
Expers. 10–20											
Aver. dif.§		−2.24	+0.77		−0.60	−0.52		−0.75	−0.47	−0.43	−0.45
S. E.		0.367	0.449		0.110	0.077		0.100	0.095	0.055	0.055
p		<0.001	>0.1		<0.001	<0.001		<0.001	<0.01	<0.001	<0.001
Expers. 10–20											
Aver. dif.**			+3.01			+0.08				+0.32	−0.02††
S. E.			0.582			0.110				0.095	0.055
p			<0.001			>0.4				<0.01	>0.7

* Forearm venous volume which would have existed during air hyperventilation if venous pressure had not changed from the resting value.
† Forearm venous volume which would have existed during CO_2 hyperventilation if venous pressure had not changed from the resting value.
‡ Standard deviation.
§ Average difference between resting and hyperventilation values.
‖ Standard error.
¶ Probability.
** Average difference between air hyperventilation and CO_2 hyperventilation values.
†† Average difference between the venous volume change with air hyperventilation and with CO_2 hyperventilation if venous pressure had not changed from the resting value.

tissue. This volume decreased in each instance during hyperventilation while breathing air. The average decrease was 0.89 ml. per 100 ml. ($p < 0.001$) or 30.9 per cent of the average resting venous volume of the forearm. The reduction in volume which would have occurred if there had been only venous contriction and no change in venous pressure averaged 0.68 ml. per 100 ml. ($p < 0.001$) or 23.6 per cent of the average resting value. The reduction which would have occurred if there had been only a fall in venous pressure and no change in venous tone averaged 0.21 ml. per 100 ml. or 7.3 per cent of the resting value.

In the final 11 experiments the resting volume averaged 2.65 ml. per 100 ml. The average decrease was 0.75 ml. per 100 ml. while overbreathing air and 0.43 ml. per 100 ml. while overbreathing 5 or 7 per cent CO_2. The reduction in volume averaged 0.32 ml. per 100 ml. less while overbreathing 5 or 7 per cent CO_2 than while overbreathing air. This difference in response was significant ($p < 0.01$) and was attributed to the failure of venous pressure to fall while overbreathing CO_2. The venous volume decrease caused by venous constriction alone while overbreathing air averaged 0.47 ml. per 100 ml. and was not significantly different ($p > 0.7$) from the average decrease of 0.45 ml. per 100 ml. observed while overbreathing CO_2.

DISCUSSION

Burnum, Hickam, and McIntosh (14) found an appreciable increase in cardiac output during voluntary hyperventilation. This increase would require a pressure-volume readjustment within the venous system which would increase the availability of blood to the heart. It might be accomplished, at least in part, by peripheral venous constriction and shift of blood centrally. Recent studies (8, 10, 15) indicate that the veins are capable of active constriction to the extent that large amounts of blood may be moved from the limbs even in the face of an increased venous distending pressure. In the experiments reported here hyperventilation while breathing air was associated with shifts of blood from the limbs which averaged 30.9 per cent of the average resting venous volume of the forearm. Changes in volume attributable to active venous constriction alone averaged 23.6 per cent of the average resting forearm venous volume. Such shifts of blood from four extremities, if redistributed toward the heart, certainly would be consistent with an increased cardiac output.

Recent work strengthens the suggestion that peripheral venous constriction is an important component of the circulatory changes which occur during hyperventilation. Weissler, Leonard, and Warren (16) found the cardiac output to increase in recumbent subjects when tachycardia was induced by atropine. This response to atropine was significantly less when subjects were in the 60 degree upright position. The decreased response was attributed to pooling of blood in dependent parts of the body with resulting depletion of the central venous reservoir and a reduction in the amount of blood available to the heart. Gleason, Berry, Mauney, and McIntosh (17) observed that the increased cardiac output during hyperventilation was proportionate to the increase in heart rate in both the recumbent and the upright subject. These observations suggest that hyperventilation in the upright position is associated with a more adequate central venous reservoir. Peripheral venous constriction may serve to maintain or increase the availability of blood to the heart during overbreathing.

In our experiments venous constriction occurred to about the same extent during hyperventilation while breathing either air or 5 or 7 per cent CO_2. However, peripheral venous pressure did not fall while overbreathing CO_2 as it did while overbreathing air. This failure of venous pressure to fall accounts for the smaller shift of blood from the limbs when the inspired gas contained a high concentration of CO_2.

SUMMARY

Forearm venous distensibility, pressure and volume were measured before and during voluntary hyperventilation in 20 experiments. In 11 of these experiments the measurements were repeated during voluntary hyperventilation with 5 or 7 per cent CO_2 in the inspired gas. The following observations were made:

1. Forearm venous pressure decreased during hyperventilation while breathing air and averaged

a slight increase during hyperventilation while breathing CO_2.

2. Active forearm venous constriction occurred regularly during hyperventilation. The response observed while overbreathing air was not significantly different from that observed while overbreathing CO_2.

3. Blood shifted out of the forearm veins in each instance during hyperventilation while breathing air. This decrease in forearm venous volume averaged 30.9 per cent; the decrease attributable to venous constriction alone averaged 23.6 per cent; and the decrease caused by the fall in venous pressure alone averaged 7.3 per cent of the average resting value.

4. Blood also shifted from the forearm veins during hyperventilation while breathing CO_2. However, the volume change was significantly less than while overbreathing air because forearm venous pressure failed to fall.

5. It is suggested that peripheral venous constriction may serve to increase the availability of blood to the heart during hyperventilation.

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THE EFFECT OF INFLAMMATION ON THE HEXURONATE-CONTAINING POLYSACCHARIDES OF HUMAN PLASMA[1]

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The observation has been made (1) that polysaccharides containing uronic acid are increased in the plasma of patients with rheumatoid arthritis. In view of the probable identity of these polysaccharides with some components of ground substance (2–4), this is an observation of considerable interest. If it should apply only to patients with rheumatoid arthritis, it might be a reflection of basic changes in the metabolism of connective tissue in that disease. The present study was done to compare in this regard patients with rheumatoid arthritis and patients with inflammatory disease unrelated to rheumatoid arthritis. Increased levels of hexuronate-containing polysaccharides were observed in plasma from patients with rheumatoid arthritis. However similar increases were noted in the presence of nonrheumatoid inflammatory states.

EXPERIMENTS

Oxalated blood for plasma was obtained freshly from three groups of individuals: Group A, healthy ambulatory individuals and hospitalized patients with no evidence of inflammatory, hematologic, neoplastic, primary metabolic or connective tissue disease; Group B, hospitalized patients with no evidence of hematologic, neoplastic, primary metabolic or connective tissue disease, but with significant inflammatory reactions, for the most part either post-traumatic or secondary to pulmonary or urinary tract infection; and Group C, hospitalized or clinic patients with clinically unequivocal active rheumatoid arthritis, without regard to stage or grade. No patients were receiving adrenocorticosteroids. Most of the rheumatoid patients were on salicylates. C-reactive protein tests were negative in all individuals in Group A and positive in all patients in Groups B and C. With few exceptions, blood samples were obtained in fasting periods or at least three hours postprandially. Additional healthy laboratory personnel were studied before, during and after five day periods of salicylate ingestion in dosage sufficient to induce continuous mild to moderate symptoms of salicylism. C-reactive protein tests were negative throughout these studies except for a transient positive reaction in one individual, occurring during the period of salicylate ingestion and coincident with the occurrence of slight tenderness at site of a venipuncture.

Two series of observations were made, each series comprising three groups of patients as just defined. In Series I, euglobulins were precipitated by the mineral acid dilution method of Erickson, Volkin, Craig, Cooper, and Neurath (5) from duplicate 1 ml. and duplicate 5 ml. samples of plasma, to each of which a quantity of physiologic saline equal to one-half of the original plasma volume had been added. The 1 ml. samples were taken up to original plasma volume in alkaline saline, and protein content was determined by biuret reaction (6). Acid mucopolysaccharides were recovered from the 5 ml. samples by a previously described method (4) involving hydrolysis of proteins with crystalline pepsin and trypsin, dialysis of hydrolysates, and then precipitation of acid mucopolysaccharides with quarternary ammonium bromide. In Series II, acid mucopolysaccharides were recovered from duplicate 5.5 ml. samples of plasma by the method of Bollet, Seraydarian, and Simpson (3). Biuret determinations were done on 0.5 ml. aliquots removed just prior to precipitation with perchloric acid. In both series, hexuronates were measured by the carbazole method (7).

As noted previously (4), the method of Series I yields a polysaccharide fraction in which mucoprotein contamination is evidenced by disproportionately high yields of hexosamine. Consequently the data obtained by Series I are presented for group comparison with Series II and not for absolute levels of hexuronate. The method of Series II has already been noted by Bollet and associates (3) to show no evidence of mucoprotein contamination. The accurate recovery of added chondroitin sulfate (10 μg. added to 5.5 ml. of plasma) recorded by Bollet and coworkers was confirmed, as was the degree of accuracy possible with the method. Duplicate samples checked within 10 per cent in both series.

RESULTS

Samples of the material recovered were compared on paper chromatography with commercial chondroitin sulfate (General Biochemicals, Inc.) and with heparin (Liquaemin®, Organon, Inc.), using methods previously described (8). Metachromasia developed with toluidine blue. Mobility of the two components observed was identical with

[1] This work was supported by grants from the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service, and the American Heart Association.

TABLE I

Hexuronate-containing polysaccharide levels in the euglobulin fraction of human plasma, recovered by Method I and measured by hexuronic acid content *

Subject groups (no. in group)	Euglobulins (mg.) per 100 ml. plasma	Hexuronic acid (μg.)	
		Per 100 ml. plasma	Per 100 mg. euglobulin
A—Noninflammatory 23	411 ± 94 (S. D.)	226 ± 75	56 ± 15
B—Inflammatory 26	748 ± 126	356 ± 118	48 ± 16
C—Rheumatoid 25	702 ± 189	372 ± 117	54 ± 13

* As noted in text, hexuronic acid levels determined by Method I are presented for group comparisons only. Disproportionately high hexosamine levels (4) indicate some degree of mucoprotein contamination of the fraction measured.

that already described (4) for the acid mucopolysaccharides recovered from Cohn fractions of human plasma.

Results of the survey of the three groups of individuals in Series I are summarized in Table I. As noted in the Experiments section, the data in this series are presented for group comparisons and not for conclusions regarding absolute levels of acid mucopolysaccharides.

Results of the survey of another three groups of individuals by the method of Bollet and associates (Series II) are presented in Table II. The protein levels were determined prior to perchloric acid precipitation.

In both series, the euglobulin fraction in Groups B and C was significantly greater than in Group A. This has been observed consistently in previous studies (9) and represents a nonspecific change found in any patient with an active inflammatory process. In both series the level of nondialyzable polysaccharides as measured by hexuronate content was higher in Groups B and C than in Group A. In Series I, the elevation was highly significant ($p < 0.01$) on comparison of Groups B and C with Group A. In Series II the elevation was highly significant ($p < 0.01$) on comparison of Group B with Group A and possibly significant ($p < 0.05$) on comparison of Group C with Group A.

The plasma levels of acid mucopolysaccharides

TABLE II

Plasma levels of hexuronate-containing polysaccharides, recovered by Method II (3) and measured by hexuronic acid content

Individual subjects	Group A Noninflammatory		Group B Inflammatory		Group C Rheumatoids	
	Euglobulins (mg.) per 100 ml. plasma*	Hexuronic acid (μg.) per 100 ml. plasma	Euglobulins (mg.) per 100 ml. plasma*	Hexuronic acid (μg.) per 100 ml. plasma	Euglobulins (mg.) per 100 ml. plasma*	Hexuronic acid (μg.) per 100 ml. plasma
1	643	152	802	207	1,090	181
2	544	157	923	216	1,020	148
3	615	163	1,061	178	932	148
4	632	171	878	231	915	159
5	421	157	1,361	165	783	224
6	307	135	895	229	829	212
7	643	121	1,061	173	999	253
8	623	120	1,072	177	736	166
9	706	118	969	252	669	151
10	857	127	1,021	122	1,094	235
11	596	159	1,227	177	906	145
12	570	168	1,118	129	1,062	123
13	809	118	1,320	122	1,411	137
Average of group (± S. D.)	613 ± 143	144 ± 21	1,054 ± 169	183 ± 43	957 ± 192	176 ± 42

* Before perchloric acid precipitation.

of subjects in Group A of Series II were considerably lower than the serum levels of normal individuals as reported by Bollet and co-workers (3).

By Method I, mild salicylism for five days in normal individuals produced no consistent change in amount of acid mucopolysaccharides recovered from plasma proteins.

DISCUSSION

The level of acid mucopolysaccharides recovered from plasma proteins of patients with rheumatoid arthritis was found to be increased as compared to a control group. This observation is in accord with an earlier report by Badin, Schubert, and Vouras (1). On evaluating a group of patients with a comparable degree of inflammation unrelated to rheumatoid disease, the level of acid mucopolysaccharides in plasma was found to be similarly increased on comparison with the control group. It is of interest that the increase in Series II (where mucoprotein contamination was not a factor) was more consistent in patients with nonrheumatoid inflammatory states than in the rheumatoid group. The increase in both instances paralleled fairly closely the increase in plasma euglobulins which was noted in the presence of any inflammatory process, including rheumatoid arthritis. Thus at present, the acid mucopolysaccharides must be classified with the numerous other substances known to occur in increased amounts in the plasma of patients with inflammatory processes in general. As a material whose study may elucidate some of the factors involved in the combination of acid mucopolysaccharides with protein, it offers promise, nonetheless. The striking difference between serum levels of acid mucopolysaccharides as reported by Bollet and associates (3) and plasma levels as noted in the present study is under further investigation now.

SUMMARY

The acid mucopolysaccharides recoverable from the euglobulin fraction of human plasma were found to be increased in patients with rheumatoid arthritis as compared to a normal group. The increase was nonspecific in that it occurred to an equal degree in patients with inflammatory processes unrelated to rheumatoid disease. The increase in acid mucopolysaccharides in inflammatory processes, both rheumatoid and nonrheumatoid, was accompanied by an increase in the euglobulin fraction of plasma from which the acid mucopolysaccharides were recovered.

ACKNOWLEDGMENT

The technical assistance of Mrs. Nancy Langley is gratefully acknowledged.

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THE FIBRINOLYTIC ENZYME SYSTEM IN NORMAL, HEMORRHAGIC AND DISEASE STATES[1]

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The recognition that hypofibrinogenemia may be an important factor in the hemorrhagic diathesis which occurs in a number of pathological states has given rise to an increased study of this phenomenon. In many of these conditions an accompanying fibrinolysin has been reported. However, evidence as to the presence of this enzyme varies from investigator to investigator and from method to method.

Therefore, a study has been undertaken to investigate as quantitatively as possible the various factors of the fibrinolytic enzyme system. Early in the investigation it became evident that an active fibrinolysin could be detected only transiently in the blood and that a large part of the discrepancy found in the literature may have been a result of the particular time at which the sample was drawn. It has been frequently observed that lysis of plasma clots at all dilutions may occur at one stage, and yet a blood sample drawn an hour later, which has a lower fibrinogen level, may show no evidence of lysis. Indirect evidence obtained from a changing profibrinolysin or inhibitor level may, therefore, give more information as to what is occurring intravascularly than does the presence or absence of an active fibrinolysis.

The following set of experimental tests has been set up as a routine for examining blood from patients with hemorrhagic tendencies which are suspected to be in the field of hypofibrinogenemia or fibrinolysin. These tests have also been applied to a number of normal subjects, pregnant women, obstetrical patients with abruptio placentae, patients undergoing portacaval shunt for cirrhosis of the liver, as well as patients with carcinoma and other disease states.

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METHODS

Fibrinogen determinations are made by the method of Ratnoff and Menzie (1).

Plasma profibrinolysin, active and "free" (streptokinase-activatable, in the presence of plasma inhibitors). This is a method similar to that of Scott, Matthews, Butterworth, and Frommeyer (2) but the details have been worked out independently in this laboratory and have been reported previously (3). However, since earlier reports, it has been found necessary to change to a casein substrate since the commercial fibrinogen is no longer suitable for these studies (presumably because of the presence of large quantities of profibrinolysin). Although this determination now represents the hydrolysis of casein rather than the more specific fibrinogen molecule, the original term has been retained.

Into each of three conical centrifuge tubes containing 0.9, 0.9, and 0.85 ml., respectively, of 0.05 M phosphate buffer, pH 7.4, 0.1 ml. portions of the plasma to be tested are pipetted. To Tube 3 is added 0.05 ml. of streptokinase [2] (10,000 μ per ml.). One ml. of 1 per cent casein solution is then added and the proteins of Tube 1 are immediately precipitated with 3 ml. of 5 per cent trichloracetic acid. The other tubes are then incubated in a water bath at 37° C. for two hours before hydrolysis is stopped and the proteins are precipitated with trichloracetic acid. After standing one hour, the tubes are centrifuged and the optical density of the supernatant solution is measured in a Beckman spectrophotometer using as a blank a solution prepared by mixing casein, buffer and trichloracetic acid in the proportions given above.

The difference in optical density between the supernatant of Tubes 1 and 2 is a measure of the active proteolytic enzyme found in the plasma. Only in rare instances is significant activity demonstrable. The difference in optical density between Tubes 1 and 3 is an indication of the active plus the profibrinolysin which can be activated by means of streptokinase. The actual units of fibrinolytic activity can then be read from a previously prepared calibration curve relating optical density to units of activity (4). The units of active enzyme should be subtracted from those obtained after addition of streptokinase in order to determine the profibrinolysin. However, since inhibitors of the enzyme are present in

[2] Varidase® from Lederle Laboratories Division, American Cyanamid Company.

the plasma, not all of the activated enzyme is available for hydrolysis. The plasma profibrinolysin then represents only that which is not inhibited and will be called hereafter "free" profibrinolysin.

Total active and profibrinolysin. In order to obtain the uninhibited enzyme, the inhibitors are removed by precipitating the fibrinolytic enzyme at pH 5.1 and low ionic strength as first described by Milstone (5). Plasma (0.5 ml.) is diluted 1 to 20 with distilled water, and 0.15 ml. of 1 per cent acetic acid is added. The solution is allowed to stand overnight in the refrigerator and then centrifuged. The supernatant containing the inhibitors is discarded and the precipitate is dissolved in 0.5 ml. of phosphate buffer. This solution is then tested in the same manner as was the plasma for active and streptokinase-activatable profibrinolysin. Any active enzyme found in this euglobulin precipitate must be subtracted from that found after activating with streptokinase to obtain the *total* profibrinolysin. This calculation should be made after optical density has been converted into units of fibrinolysin by means of the calibration curve since there is not a straight line relationship between optical density and fibrinolytic units.

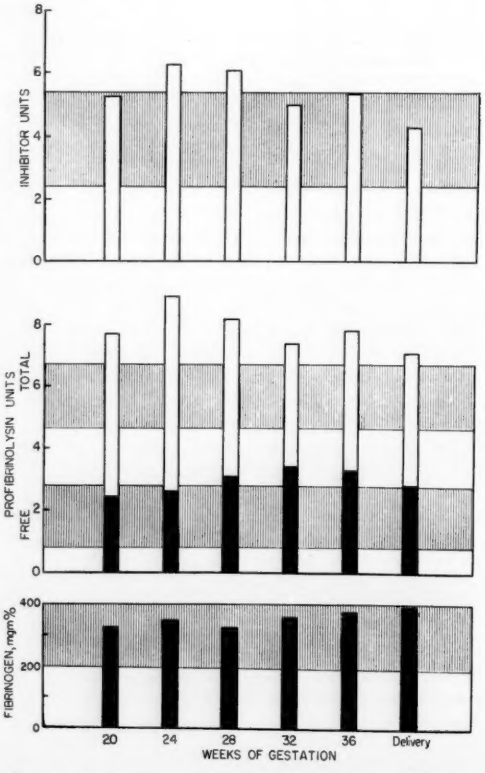

FIG. 1. FIBRINOLYTIC ENZYME ACTIVITY IN NORMAL PREGNANCY

In the cases reported here no active enzyme was found in either the plasma or euglobulin precipitate. However, since it does occur in some cases, the method and precautions concerning the calculations of profibrinolysin have been discussed in this paper.

Inhibitors. The difference between the total profibrinolysin found in the iso-electric precipitate and the free proenzyme found in the plasma is a measure of the inhibitors contained in the plasma. On rare occasions when a severe afibrinogenemia is encountered, the profibrinolysin of the iso-electric precipitate may be equal to or even less than that found in plasma. This suggests that the enzyme may not be precipitated 100 per cent but there is probably close to 90 per cent precipitation.

Modified Coon's test (6). Plasma is diluted serially with saline to a dilution of 1 to 64. The plasma is then clotted by the addition of thrombin [3] (10 units) and the clots allowed to stand at room temperature for 24 hours. The lysis is scored from minus to 6 plus, depending upon the number of tubes showing complete disappearance of the clot in 24 hours. If lysis is observed in less than 24 hours the time is recorded. Only complete disintegration of the clot (as opposed to 50 per cent lysis, as used by Coon) is reported since clot retraction makes this 50 per cent difficult to estimate.

Optical density. In addition to the above determinations, a measure of the optical densities of the trichloracetic acid (TCA) soluble products in the plasma, as determined in the Beckman spectrophotometer at 260, 280, and 295 mμ, is made. It was originally felt in this laboratory that an increase in the products indicated intravascular hydrolysis of fibrinogen. While it is still believed that a transient increase in optical density at these wave lengths may be an indication of fibrinolysis, it has become evident that these substances are also increased in a number of disease states. During pregnancy there is a slight rise which is more evident in cases of toxemia of pregnancy. In cases of advanced carcinoma these substances may be markedly increased. Acute infection, particularly where accompanied by urinary retention, may increase the levels five- to tenfold. These TCA soluble substances may be mucoproteins and/or breakdown products of nucleic acids and proteins which may occur under conditions in which increased catabolism presumably might be expected. It is recognized that optical densities, while giving straight line relationships with amino acids, need to be used with caution when carried over to fibrinolytic plasma digests or to plasma altered *in vivo*.

With the exception of the fibrinogen determination and sometimes the Coon's test these methods are too time consuming to be useful for emergency diagnosis. However, the fibrinogen level, which can be estimated qualitatively in 10 minutes or completed quantitatively in 45 minutes, is the most important determination in following the patient's condition. The other determinations, as outlined, can then be made in an effort to elucidate the processes involved in production of an acute afibrinogene-

[3] Parke Davis bovine thrombin.

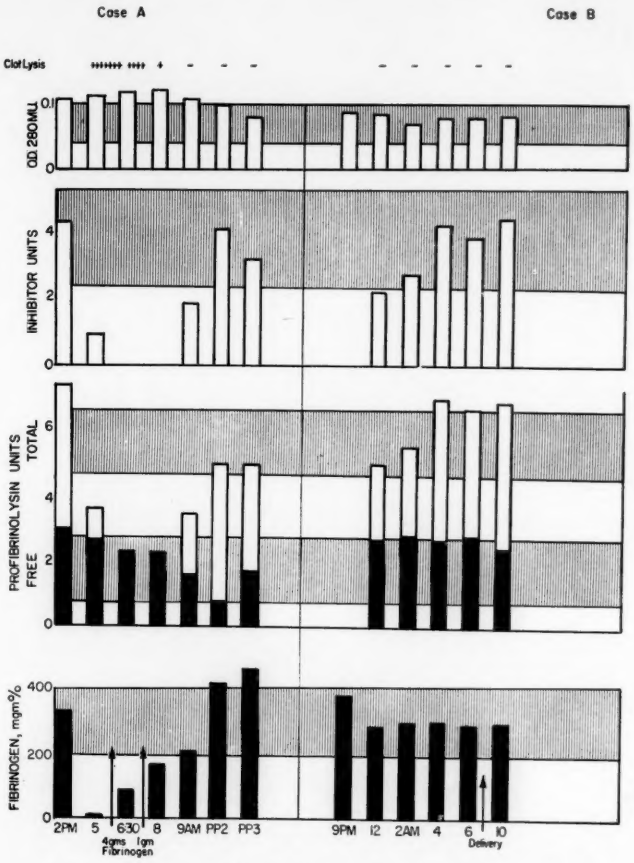

FIG. 2. FIBRINOLYTIC ENZYME ACTIVITY IN ABRUPTIO PLACENTAE

mia after the immediate crisis has passed, or to study more chronic conditions.

RESULTS

The cross hatching in the following figures represents the ranges to be expected for normal nonpregnant subjects. Two hundred to 400 mg. per cent of fibrinogen [that obtained by Ratnoff and Menzie (1)] has been used as the normal range for this substance. The ranges for other factors represent those expected in 99 per cent of the population as determined from the mean plus or minus 2.5 times the standard deviation for each factor. The results of the statistical analyses are as follows. Free profibrinolysin: Mean, 1.8; S.D., 0.4; range, 0.8 to 2.8. Total profibrinolysin: Mean, 5.7; S.D., 0.4; range, 4.7 to 6.7. Inhibitors: Mean, 3.9, S.D., 0.6; range, 2.4 to 5.4. Optical density at 280 mμ: Mean, 69; S.D., 12; range, 39 to 99.

Pregnancy

Since it is in obstetrical practice that most cases of afibrinogenemia are found, the factors of the fibrinolytic enzyme system were studied throughout pregnancy in a group of approximately 50 women with normal antepartum, delivery, and postpartum courses. In Figure 1 are charted the averages of figures obtained for these patients at approximately the period of pregnancy indicated. Not all patients are represented at each level.

The fibrinogen level rises gradually from an

average of 300 mg. per cent in the first trimester [this agrees well with normal nonpregnant figures reported by other investigators (1)] to 400 mg. per cent at term. The total profibrinolysin and inhibitor levels rise to a maximum in the second trimester and then decrease somewhat. The free profibrinolysin continues to increase into the third trimester.

The increase in fibrinogen and other clotting factors (7) has been interpreted by Fresh, Ferguson, and Lewis as a mechanism to protect the mother from hemorrhage during the forthcoming delivery. The higher free and total profibrinolysin may in turn be a protection against thrombotic complications, while the simultaneously increased inhibitors appear to hold the entire system in check. However, in spite of the rise in all factors during the second trimester, there seems to be a considerable utilization and/or destruction of both profibrinolysin and inhibitors at delivery of even a normal pregnancy. It is conceivable, then, in the case of an obstetrical accident such as abruptio placentae or fetal demise, that the process of activation and utilization of the enzyme system may be accelerated to such an extent that the intravascular fibrinogen may be completely destroyed. In such a case significant fibrinolytic activity may or may not be demonstrable depending on the time at which the blood sample is drawn.

Figure 2 shows a comparison of two patients with abruptio placentae. Case A, although showing normal levels of all factors at the time Cesarean section was performed to save the infant, developed an afibrinogenemia and severe hemorrhage approximately three hours post delivery. Five grams of fibrinogen plus whole blood replacement restored the clotting mechanism, and vital signs were stabilized within two hours after hemorrhage started. All factors of the fibrinolytic enzyme system (fibrinogen, "free" and total profibrinolysin, and inhibitors) fell precipitately during the period before hemorrhage occurred, but rose again gradually as the patient recovered.

In Case B, the fetal heart was not heard at the time of admission to the hospital and conservative treatment was therefore decided upon in spite of considerable bleeding. However, fibrinogen levels were followed carefully during the course of labor. Although there was an initial drop in fibrinogen and perhaps in other factors (the quantity of blood obtained in the first sample was insufficient for determinations other than fibrinogen), the patient stabilized at levels well within normal range of fibrinogen and total profibrinolysin, and the inhibitors increased from low normal levels to well within the average range. A low forceps delivery of a stillborn infant was accomplished without undue incident approximately eight hours after the original diagnosis of abruptio placentae was made.

These two cases are presented because they show so clearly the parallelism between fibrinogen levels on the one hand and the profibrinolysin and inhibitor levels on the other. Whenever a severe case of afibrinogenemia is encountered in obstetrics, the levels of profibrinolysin and inhibitors are also low. However, even in cases of abruptio placentae when profibrinolysin and inhibitors remain within normal limits, the fibrinogen usually is also found to be adequate for normal clotting.

Cirrhosis of liver

In Figure 3 results are presented of determinations on blood samples from two patients on whom portacaval shunts were performed for cirrhosis of the liver. In Case C, the first sample was drawn at completion of the anastomosis when generalized oozing from all cut surfaces was becoming an acute problem. The fibrinogen level was somewhat low (168 mg. per cent) as were the other factors of the fibrinolytic system, although these were typical of the levels found in cirrhotic patients. A 6 plus lysis of plasma clots occurred within one hour. The patient was losing nearly one liter of blood every 15 minutes. This was being replaced with whole blood. In spite of 8 grams of fibrinogen, the level continued to fall (46 mg. per cent) and the patient was returned to the operating room for re-exploration. A small puncture in the vena cava was found and repaired and more blood and fibrinogen were administered. Although the fibrinogen level rose somewhat, total profibrinolysin and inhibitors continued to decrease and the patient expired approximately six hours after the completion of the original operation.

Case D shows results obtained before and during the operation on another cirrhotic patient. On admission one week before the operation, this patient's levels (except for fibrinogen) were essentially the same as those found in the initial de-

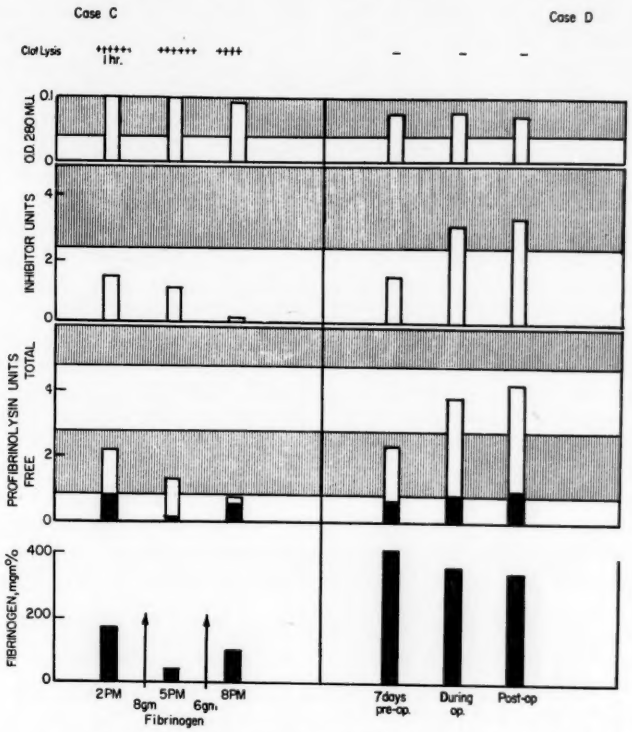

FIG. 3. FIBRINOLYTIC ENZYME ACTIVITY IN CIRRHOSIS OF LIVER

termination of Case C. However, after preoperative administration of albumin, the total profibrinolysin and inhibitors had risen to nearly normal and a portacaval shunt was performed on this patient without difficulty.

Other pathological processes

In Figure 4, the results obtained with normal subjects are contrasted with three groups of pathological patients: *A*) Averages obtained from patients with benign prostatic hypertrophy and with carcinoma of the prostate; *B*) Typical cases of early and advanced carcinoma of the cervix; and *C*) A case of severe septic abortion.

No cases of hypofibrinogenemia were encountered in this series of cases with prostatic carcinoma. In fact, averages for fibrinogen figures were found to be higher than those of normal controls and of those with benign prostatic hypertrophy. Other factors of the fibrinolytic system were within normal limits, except for the free profibrinolysin which showed somewhat high averages. However, optical densities of most patients with prostatic carcinoma were found to be considerably elevated. Similar results were obtained with advanced cervical cancer except that frequently an exceedingly high fibrinogen level was encountered. Early cancer of the cervix showed perfectly normal levels for all factors.

Although fibrinogen levels may vary widely in cases of severe infection, they tend to be somewhat high as shown in Figure 4. The "free" profibrinolysin is frequently exceedingly low with the total profibrinolysin in a low normal or slightly below normal range. The inhibitors usually remain within normal limits. High optical densities may also be encountered when infection is overwhelming.

DISCUSSION

The fibrinolytic enzyme system is a complicated system consisting of proenzyme(s), activators, ac-

tive enzyme(s), inhibitors and substrates. Because of the complexity of factors involved, it is difficult to determine whether one or more substances are involved in each step of the hydrolysis process. However, the confusion found in the literature as to the mode of action and importance of this enzyme in afibrinogenemia suggests that many investigators are studying different aspects of the enzyme system. An excellent review article has recently been published by Astrup (8) which describes in detail all the complexities encountered in a study of the enzyme.

Tissue activators have been found to exist in almost all tissues, primarily in the mitochondria and microsome fractions, and have been studied extensively by a number of investigators (4, 9, 10). It has been demonstrated that activation by tissue extracts may produce fibrinogenolytic activity but not fibrinolytic activity, or vice versa (4). This is substantiated by the fact that occasionally plasma clots will be lysed in samples where no caseinolytic activity can be demonstrated, or that caseinolytic activity is encountered when no clot lysis occurs.

Tissue activation is the most probable pathway encountered *in vivo,* but the speed and ease of activation by streptokinase makes it the usual method of choice for *in vitro* studies.

Inhibitors to both the activation process and the hydrolysis process probably are present in the plasma, and different methods of determination probably measure different substances. One set

FIGURE IV

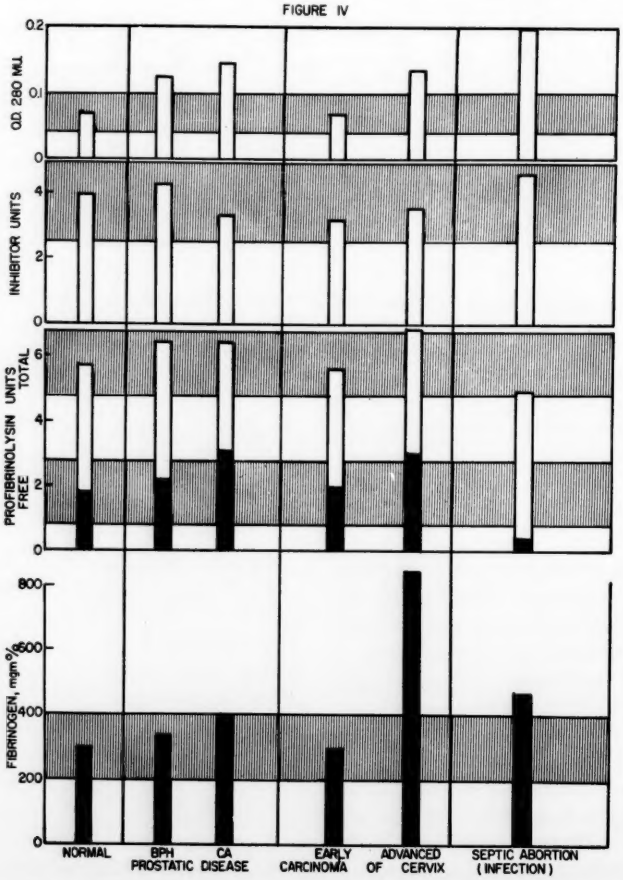

FIG. 4. FIBRINOLYTIC ENZYME ACTIVITY IN A VARIETY OF DISEASES

of inhibitors is generally believed to be associated with the albumin fraction of plasma. Therefore, an iso-electric precipitation of the enzyme at low ionic strength should remove these inhibitory factors. However, while it is recognized that all the possible inhibitors may not be removed (11, 12), nevertheless, they are substantially reduced by this procedure. Shulman (13) has shown evidence to suggest that fibrinogen itself appears to produce an inhibitory effect on fibrinolysin.

While fibrinolysin is usually thought of as acting only on fibrinogen and fibrin, it must be remembered that it is a general proteolytic enzyme. Although albumin and globulin do not appear to be destroyed by this enzyme, other constituents of the plasma may be. Recently, there have been reports of the destruction of clotting factors other than fibrinogen by this enzyme (14–16). Thus proaccelerin, platelets, antihemophilic globulin, and perhaps prothrombin, may all be hydrolyzed by fibrinolysin. Therefore, multiple clotting deficiencies may result from the activation of the fibrinolytic enzyme. The methods presented here, while measuring only a few of the numerous factors, do give a comprehensive picture of the processes involved.

The active fibrinolysin is an extremely labile enzyme which is apparently rapidly denatured both *in vivo* and *in vitro*. Although rapid clot lysis was observed in some samples of blood drawn in Cases A and C presented here, no significant active proteolysis was demonstrable. However, the fact that the total profibrinolysin was considerably reduced in both of these cases is strong suggestive evidence that the precursor has been activated and then denatured after it has removed the fibrinogen from the blood stream by hydrolysis. Conversely, fibrinogen levels remained normal in Cases B and D, where profibrinolysin levels also stayed within a normal range, even under such conditions of stress as abruptio placentae and extensive surgical procedures.

It is suggested that in cases of afibrinogenemia, inhibitors of the activation process may first be reduced, perhaps by adsorption on the microsomes of tissue extract as suggested by Astrup (17). This removal of inhibitors may permit a portion of the profibrinolysin to be activated, either by enzymes of the tissue extract or by some other activator present in the blood stream as suggested by Troll and Sherry (18). The proteolytic enzyme thus produced may then hydrolyze the fibrinogen and other clotting factors and perhaps cause the removal of more inhibitors. The whole process may then be repeated until a severe afibrinogenemia is encountered, which can be controlled only by the intravenous administration of fibrinogen. Since the active enzyme is rapidly destroyed, if sufficient fibrinogen is given to control the hemorrhage for a short period of time the procedure may then be reversed, as the patient's own protective mechanisms start to resynthesize the various factors involved, *i.e.*, fibrinogen, inhibitors, and profibrinolysin.

The data presented on patients throughout pregnancy give an overall view of what may be expected to occur during normal pregnancy. Where many factors of the system vary from these normal levels, it may be supposed that there is an interdependence of these factors on each other. Johnson and Tillett (19) have shown that streptokinase injected into the blood stream of rabbits produced an active lytic enzyme accompanied by a moderate fall in serum inhibitors and fibrinogen and a striking fall in plasminogen (profibrinolysin). Lewis and Ferguson, using staphylokinase and fibrinolysin, obtained similar results in dogs (20).

The similarity in results in the two cases of severe hemorrhage from afibrinogenemia with those reported by the previously quoted authors, makes it seem probable that the same mechanisms were involved. The time required for afibrinogenemia to develop after Cesarean section in Case A (three hours) makes an enzymatic process of removal of fibrinogen more probable than a mechanism of intravascular defibrination by clotting. This case also negates the idea prevalent among obstetricians that afibrinogenemia will not develop once the uterus is emptied.

Case C emphasized the evidence previously presented from this laboratory (21) that even slightly decreased fibrinogen levels (125 to 175 mg. per cent) may be dangerous in the surgical patient and demonstrates that rapid plasma clot lysis may sometimes be a more effective test of a dangerous clotting defect than is the fibrinogen level.

The first enzyme levels found in both Cases C and D, which are typical of cirrhotic cases, suggest that the hemorrhagic tendencies of these pa-

tients may be at least partially due to fibrinolysin and sometimes to hypofibrinogenemia. The chronically low levels of profibrinolysin suggest that the enzyme is slowly but constantly going through a process of activation, utilization and destruction. The low inhibitors in these cases may be associated with the low serum albumin levels usually observed in cirrhotic patients. The results presented in Case D also point out the efficacy of giving serum albumin prophylactically, not only to restore the normal albumin-globulin ratio, but also to raise inhibitor levels.

The evidence presented here shows that advanced carcinoma is more apt to be accompanied by high levels of fibrinogen and profibrinolysin than low levels. This is in spite of the evidence presented by Tagnon (*22*) that a 10 to 12 per cent incidence of fibrinolysin and hypofibrinogenemia may be expected in cases of metastatic carcinoma of the prostate. However, these high levels may have been due to the fact that most of the patients had been on stilbesterol for some time before they were studied (*23*). Extremely high optical densities of TCA soluble products have been observed in most cases of advanced cancer. Severe infection is also apt to produce high levels of fibrinogen and optical density although the profibrinolysin is at low normal levels.

While the differences obtained between normal and cancer patients are not highly significant in most determinations except optical densities, the characteristic picture of high fibrinogen, high profibrinolysin, and high optical densities is found in cases of carcinoma and pregnancy while infection produces high fibrinogen and high optical densities. These are the same three categories in which so many of the "diagnostic tests" for cancer give similar positive results. Unfortunately, as with so many of the tests for cancer, these results vary only slightly from possible normal ranges and they are observed primarily only in cases of advanced carcinoma where the disease has usually already made itself evident in other ways.

SUMMARY

A group of methods for investigating the factors of the fibrinolytic enzyme system in human plasma is presented. These factors include fibrinogen, active fibrinolysin, "free" and total profibrinolysin, and inhibitors. This set of determinations gives a more complete indication of changes taking place in the system than do the one or two assays usually employed for determination of fibrinolysis.

Normal obstetrical patients have been followed throughout pregnancy in order to establish base lines for comparison with cases of obstetrical complications. Normal nonpregnant levels are compared with cases involving surgical hemorrhage and disease states.

A study of these factors in cases of hemorrhage following abruptio placentae and surgery for cirrhosis of the liver indicates that fibrinolysis is probably the principle cause of afibrinogenemia. Whenever low fibrinogen levels are encountered, the total profibrinolysin and inhibitor levels are also reduced. It is suggested that the profibrinolysin is activated in these cases, thus reducing the quantity of precursor. The active fibrinolysin so formed destroys the fibrinogen by intravascular hydrolysis and is itself then rapidly denatured. The lability of the active enzyme makes it difficult to detect *in vitro*.

Many disease states such as cirrhosis of the liver, advanced carcinoma, benign prostatic hypertrophy and infection appear to present their own pattern of variation in the fibrinolytic enzyme system. These changes are probably secondary in nature.

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EFFECT OF BLOOD STORAGE CONDITIONS ON THE ELECTROPHORETIC PATTERNS OF RED CELL HEMOLYSATES [1]

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During the storage of acid-citrate-dextrose (ACD) blood in a cotton-plugged flask, the electrophoretic components of the red cell hemolysate undergo changes in concentration (1). Indirect evidence indicated that the decrease in the slowest moving boundary, designated as Component B, might be correlated with the viability of the red blood cell. The standard blood bank procedures now in use involve collection and storage of blood in glass bottles or in plastic bags (closed systems). Studies were undertaken to compare the changes in the concentration of Component B in hemolysates of blood stored under a variety of conditions.

METHODS

The general procedures followed for the collection of blood in ACD and for electrophoresis have been described (1). In the present experiments, the blood was cooled by collecting the sample in a container immersed in ice water, and the sample was immediately placed in the cold room at 4° C. The 50 and 100 ml. plastic bags (EX1226) used for storage of blood were kindly provided by the Cutter Laboratories. In one case, blood was drawn directly into a bag containing ACD (NIH Sol. B); in the remaining instances blood was collected in a 125 ml. Pyrex flask or in a standard glass collecting bottle, and aliquots were transferred to the plastic bag. Storage, centrifugation, washing, lysing, clarification and dialysis against the cacodylate buffer were all conducted in the cold room.

In the previous study (1) Drabkin's procedure (2), which was used for hemolyzing the washed red cells, was found to be time consuming and yielded a solution which was altered in color due to a dark pigment. Several methods for lysing red cells were studied, which included the use of freezing and thawing (3), water and toluene, CO_2 saturated water (4), and distilled water. The patterns of red cell hemolysates obtained by freezing and thawing red cells were abnormal and contained at least six components. The three remaining procedures yielded identical patterns. Since water alone was efficient and simple to use, it was adopted as the lysing agent in the procedure used.

The samples of stored bloods were not disturbed except when aliquots were drawn. The plastic bags containing blood were each kept in an open wide-mouth bottle. Samples were stored in ice water (0° C.), on the shelf of the cold room (4° C.), or in a constant temperature bath maintained at 8° C. One sample was stirred by rotating an inclined cotton-plugged Pyrex flask continuously at 2 rpm.

After centrifugation of an aliquot, the plasma and buffy coat were removed, and the red cells were washed three times with 0.85 per cent NaCl. Lysis was accomplished by adding five volumes H_2O to one volume of red cells and shaking vigorously at intervals during a 30 minute period. The hemolysate was clarified by centrifugation for 30 minutes at 10,000 rpm in a Servall SS-1 centrifuge. The hemoglobin concentration was determined (5), and 5 ml. of a 1 per cent solution was prepared for analysis. This solution was dialyzed against 0.05 M sodium cacodylate-cacodylic acid buffer (pH 6.5) which was changed three times during an 18 to 24 hour period.

Electrophoresis proceeded for 90 minutes with closed electrodes. A photograph was taken by Longsworth's scanning technique (6) immediately after the current was shut off (Figure 1,a). A second photograph was obtained after the refractive bar of the A boundary had cleared (Figure 1,b). In this paper, the latter pattern was used for analysis. It was observed that the component areas of plates *a* and *b* could not be compared unless they were developed under precisely the same conditions, since the planimeter values of the tracings for a given area varied considerably (as much as 20 per cent) if the density of the plates differed. However, the percentage area of a given component in terms of the total pattern is comparable, regardless of the densities of the photographic plates.

Previously, Component B was delineated by dropping a line at the minimum between boundaries (1). For reasons discussed below, this procedure appeared to be unsatisfactory. It was decided to measure the area designated as B/2, which is illustrated in Figure 2. It will be noted that the partitioning line is dropped from the peak of the B boundary to the base line, and the area of the trailing material is measured by planimetry.

Inosine, purchased from the Schwarz Laboratories, Inc., was dissolved in saline, autoclaved and added to ACD blood so that a final concentration of 2,500 micromoles per 100 ml. red cells was attained.

[1] This investigation was supported by a research grant from the Surgeon General of the Army, DA-49-007-MD-762.

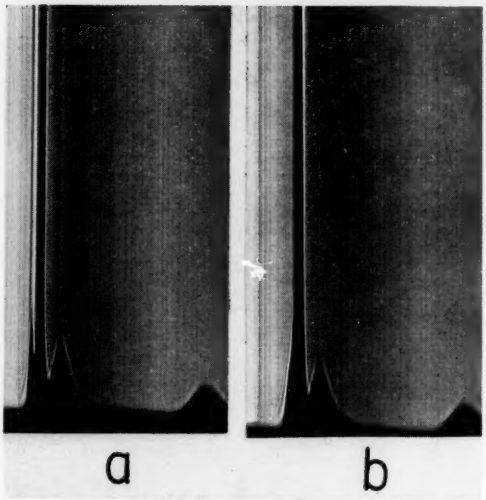

FIG. 1. ELECTROPHORETIC PATTERNS OF A RED CELL HEMOLYSATE (a) IMMEDIATELY AFTER SHUTTING OFF THE CURRENT AND (b) AFTER DIFFUSION

RESULTS

Detailed changes during storage

Patterns of red cell hemolysates of a blood sample stored for varying periods are shown in Figure 2. The changes in *a*) the B/2 concentrations, *b*) the shapes of the envelopes of Components A and B, and *c*) the mobilities of these boundaries are readily observed. A marked trailing of the B/2 area is seen in the 0 and 26 day old samples. Subsequently, the range of mobility of the B/2 material decreases and reaches a limiting value after 35 days of storage. During the first month, the percentage of B/2 in relation to the total pattern area remains fairly constant, but the height of Component B increases and the skewness decreases due to the sharpening of the boundary. After this time the B/2 concentration decreases. Examination of the patterns shows a pronounced increase in the mobilities of the three components during the first 46 days of storage. After storage for 56 and 76 days, it will be noted that the boundary for Component A is broadened and makes a contribution to the B/2 area.

An interpretation of the changes in the patterns during storage cannot be made until the individual components are separated and studied. The increased mobilities must be due to alteration in the net charges of the hemoglobins. There appears to be no doubt that any specified area represents a mixture of materials. Despite these difficulties,

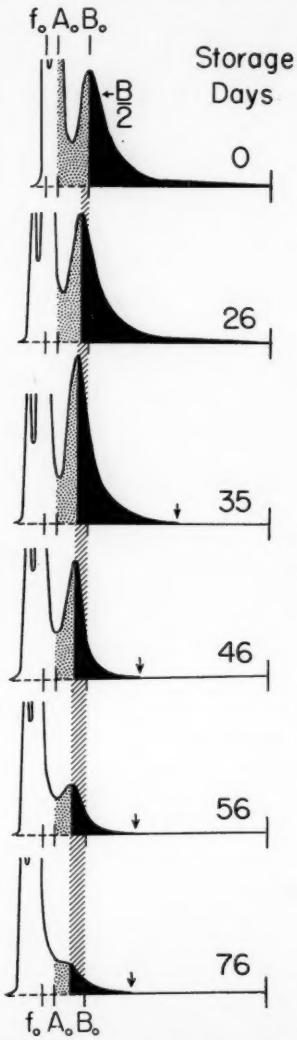

FIG. 2. ELECTROPHORETIC PATTERNS OF RED CELL HEMOLYSATES OF A BLOOD STORED FOR 76 DAYS AT 4° C. IN A COTTON-PLUGGED FLASK

The positions of the peaks for the f, A and B boundaries and for the trailing limit of the B/2 area at zero days are drawn through the base lines of each pattern. The arrow shows the position of the trailing limit of B/2 area in each pattern. The black areas represent B/2. The changes in mobility for the A and B boundaries are designated by the stippled and the lined areas.

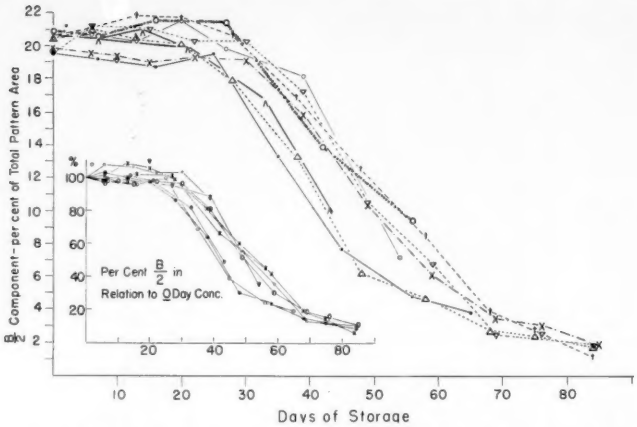

FIG. 3. CHANGES IN B/2 CONCENTRATION OF EIGHT SAMPLES OF BLOOD STORED AT 4° C. IN COTTON-PLUGGED FLASKS

a quantitative measure of B/2 area appears to serve as the best indicator for judging the overall changes occurring during storage of red cells.

Controls (Figure 3)

Blood samples drawn from five healthy young men were stored in ACD in cotton-plugged Pyrex flasks and the B/2 concentration was determined at intervals. Three samples of blood, drawn from one individual at six month intervals, yielded identical results. In another individual, two blood samples drawn at an interval of six months showed differences in the rate of decrease of the B/2 component. It is apparent that there are two families of curves in this series. A set of curves is also presented in which the results are shown as percentage concentrations of the zero day B/2 value; data were presented in this manner in a previous paper (1).

The data for the family of five curves in Figure 3 were analyzed by the method of least squares between 0 and 30 days and between 31 and 60 days of storage. The regression lines for these two periods appear to show that the B/2 concentration remains constant for at least a month of storage and that subsequently this material decreases at a steady rate. The extrapolated value of B/2 at zero concentration is 73 days. At this time, the material present in the B/2 area, which is undoubtedly a mixture of altered materials, decreases at a slower rate.

Duplicate analyses of a red cell hemolysate can be reproduced with an error of plus or minus 3 per cent. In Figure 4, the range represents a deviation of 3 per cent from the maximum and minimum values.

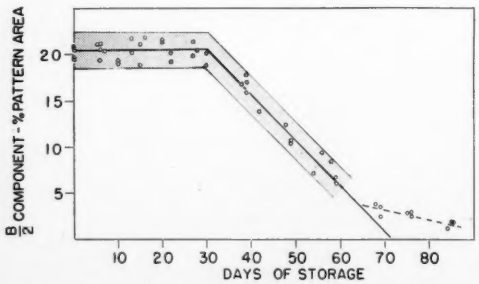

FIG. 4. A SCATTER DIAGRAM SHOWING THE REGRESSION LINES AND THE RANGE FOR THE B/2 CONCENTRATIONS OF FIVE STORED BLOODS

The stippled area is used as a standard for comparing the data presented in Figures 5 through 9.

Effect of anaerobic storage (Figures 5 and 6)

The changes in the B/2 concentration of blood stored in tightly stoppered tubes which contained no air or under mineral oil are compared with blood stored in a cotton-plugged flask. The rapid decrease in B/2 concentration during the first 10 days of anaerobic storage is striking; subsequently the values decrease at a slower rate.

Units of blood were drawn into standard glass

collecting bottles from four healthy individuals and a single sample was drawn for analysis from each bottle at varying periods of storage. It should be emphasized that a static air space is present in these bottles. The results obtained with these bloods are similar to those observed under strictly anaerobic conditions.

The changes in the B/2 concentrations of blood samples stored in plastic bags (anaerobic) were determined. In all cases the values remained within the control range for about 10 days and decreased appreciably during the next 5 days. In one experiment, 50 ml. of blood was collected in a 100 ml. bag and 50 ml. sterile air was injected so that the sample was under "aerobic" conditions during storage. In this experiment the B/2 concentration decreased in the same manner observed for the blood stored in the stoppered tubes. The rates of decrease after 20 days of storage are similar for four different blood samples.

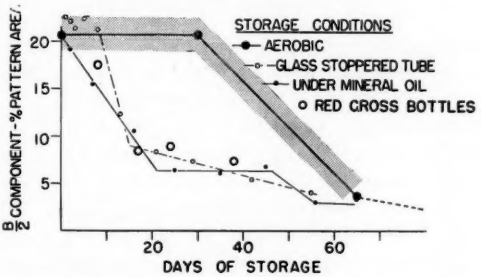

FIG. 5. CHANGES IN THE B/2 CONCENTRATIONS OF BLOOD STORED IN GLASS-STOPPERED TUBES (NO AIR SPACE), UNDER MINERAL OIL, AND IN STANDARD GLASS COLLECTION BOTTLES

Effect of storage under oxygen, nitrogen and carbon dioxide (Figure 7)

Aliquots of two blood samples were stored under conditions in which each of the three gases flowed continuously above the surface of the blood. In one group the gas was at atmospheric pressure and in the other under a slightly positive pressure (5 cm. H_2O). In both instances, the changes in the B/2 concentration were the same during storage under nitrogen or oxygen and fell within the range of values observed for blood stored in cotton-stoppered flasks. Exposure to carbon dioxide caused a rapid and marked decrease in the B/2 concentration during the first few days; the values reach a lower level in the blood stored under positive pressure.

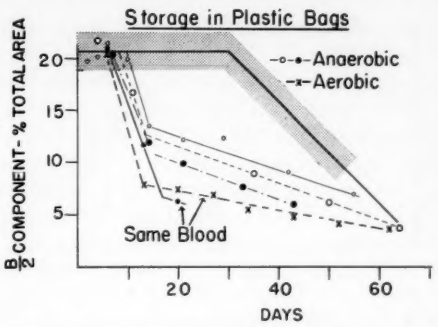

FIG. 6. EFFECT OF STORAGE IN PLASTIC BAGS ON THE B/2 CONCENTRATION

The anaerobic blood samples were stored in 50 ml. plastic bags; the aerobic samples were stored in a 100 ml. bag containing 50 ml. blood and 50 ml. air.

The pH values of the hemolysates were determined immediately after each red cell sample was lysed and clarified. The pH values for bloods exposed to CO_2 at atmospheric and slightly in-

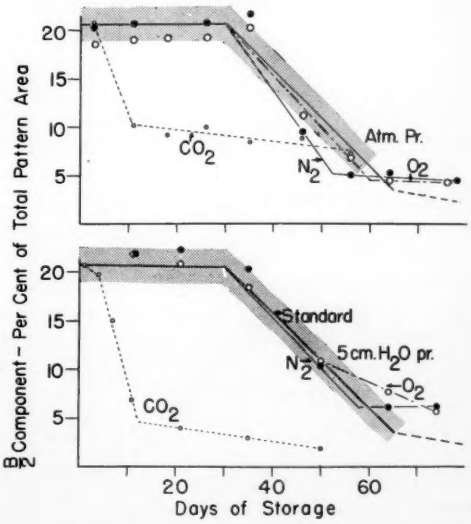

FIG. 7. EFFECT OF STORAGE UNDER OXYGEN, NITROGEN AND CARBON DIOXIDE ON THE B/2 CONCENTRATION

The gases were bubbled through water and through a sterile cotton plug before entering the flask containing the blood.

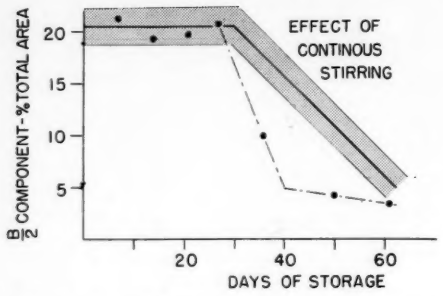

FIG. 8. EFFECT OF CONTINUOUS STIRRING ON THE B/2 CONCENTRATION

Blood in an inclined cotton-plugged flask rotated at 2 rpm.

creased pressures were 6.8 and 6.4, respectively. The oxygen and nitrogen exposed samples were at about pH 7.4.

Effect of continuous stirring (Figure 8)

Gentle stirring, accomplished by slow rotation in a cotton-plugged Pyrex flask, does not influence the B/2 concentration during the first month of storage. After this time, the rate of decrease of this material is appreciably greater than that of the control.

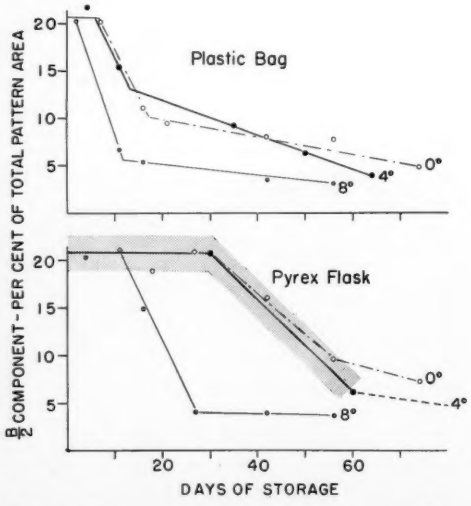

FIG. 9. EFFECT OF STORING ALIQUOTS OF THE SAME BLOOD SAMPLE AT 0, 4 AND 8° C. ON THE B/2 CONCENTRATION

Effect of temperature

Data for aliquots of a single blood sample, stored in plastic bags or in Pyrex flasks at 0, 4 and 8° C., are shown in Figure 9. No significant difference is observed for the changes in the respective 0 and 4° C. blood samples stored in plastic bags or in cotton-plugged Pyrex flasks. Identical results were obtained for a different blood sample stored at 0 and 4° in Pyrex flasks. It is remarkable that the B/2 concentrations are decreased earlier and fall to a lower level when stored under anaerobic and aerobic conditions at 8° C. These results indicate that a slight elevation in temperature has a profound influence on the composition of red cells as judged by electrophoresis.

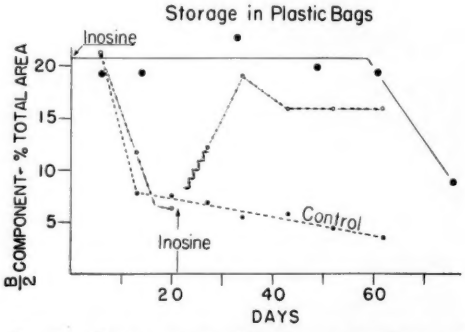

FIG. 10. EFFECT OF THE ADDITION OF INOSINE AT 0 DAY AND AFTER AN INITIAL 21 DAY PERIOD OF STORAGE IN PLASTIC BAGS ON THE B/2 CONCENTRATION

The break in the curve after adding inosine after 21 days storage represents the period of incubation.

Effect of inosine

In a previous paper (1), it was shown that the addition of inosine to blood stored in Pyrex flasks is responsible for maintaining the Component B concentration at elevated levels for a prolonged period. In view of the more rapid decrease in the B/2 concentration during storage in plastic bags, experiments were conducted to determine the effect of inosine under these conditions. The plot in Figure 10 shows that the addition of inosine at 0 days is responsible for maintaining the B/2 concentrations at the 0 day level for about 60 days. After adding inosine on the twenty-first day of storage, at which time the B/2 value is markedly depressed, a partial regeneration of this material is noted.

DISCUSSION

The changes in the concentration of the slow moving Component B/2 of red cell hemolysates during storage of ACD blood in a cotton-stoppered flask differ slightly from those reported (1). The differences are chiefly manifested in the constancy of its concentration during the early stages of storage and the subsequent steady rate in its decrease. These data which appear to be more reliable result from the following improved techniques: *a*) collection of blood, *b*) hemolysis, *c*) electrophoresis, *d*) photography, and *e*) analysis of the pattern.

It was determined that blood should be cooled immediately after it was drawn in order to avoid the changes in the electrophoretic pattern that are readily observed when samples are allowed to remain at room temperature for short periods of time. A unit of blood (550 ml.) in a standard glass collecting bottle was warmed to 37° C., and the time required for the blood to equilibrate to 4° C. in the cold room was determined under various conditions: It required five hours when the bottle stood undisturbed, three hours when the blood was mixed at frequent intervals, and one-fourth hour if the blood was mixed while the bottle was immersed in ice cold water. It is suggested that rapid cooling of blood is essential if reliable electrophoretic data for red cell hemolysates are to be obtained.

It has been noted (1) that the Component B concentration increased during the early stages of storage of blood in a cotton-plugged Pyrex flask and decreased after about 30 days. According to the data presented in this investigation for blood stored under these same conditions, the concentration of B/2 remained fairly constant for about a month and then decreased at a steady rate for about 30 days. However, it was found that the B/2 concentration decreased within a relatively short time in bloods stored under anaerobic conditions in stoppered tubes, under mineral oil, or in plastic bags. The same results were obtained in bloods stored statically in the presence of an air space in either a plastic bag or a standard glass collecting bottle. It was surprising that blood, stored under a stream of nitrogen, gave the same results for B/2 concentration that were obtained when stored under a continuous flow of O_2 or in the flask stoppered with a cotton plug. These results were in marked contrast to the almost immediate decrease in the B/2 concentration of blood stored under a continuous flow of CO_2. In view of these findings, it is assumed that the difference in the results with blood samples stored under closed and open systems are associated with the partial pressure of CO_2.

In a previous paper (1), it was suggested that the Component B concentration might serve for judging the viability of red cells. If this assumption is valid, the viability of red cells stored in a closed system would be decreased more rapidly than in an open system. Data are not yet available for correlating the electrophoretic analyses and the viability of transfused red cells.

SUMMARY

1. Standardized procedures for preparing red cell hemolysates for electrophoretic analysis and for analysis of patterns are described. Blood is cooled during collection and all procedures are conducted in the cold.

2. Detailed changes in the electrophoretic patterns of red cell hemolysates of stored blood are described. The shapes, concentrations, and mobilities of Components A and B change continually during storage. Measurement of an area designated as B/2, which represents about one-half of Component B, was used as an index of the changes occurring in the pattern during blood storage.

3. When blood is stored in a cotton-plugged flask, the B/2 component concentration remains constant for about a month and then decreases at a steady rate. The B/2 concentration decreases after about a week when blood is stored *a*) in stoppered tubes, *b*) under mineral oil, *c*) in plastic bags with and without an air space, and *d*) in standard glass collection bottles. The B/2 material begins to decrease almost immediately during exposure to CO_2.

4. The results obtained after storage of blood at 0 and 4° C. are the same. At 8° C., the B/2 concentration decreases rapidly.

5. During storage of blood under a continuous stream of O_2 or N_2, the change in B/2 concentration is similar to that observed in the cotton-plugged flask.

6. Continuous gentle stirring of blood during storage does not affect the B/2 component during the first month, but subsequently the rate of disappearance is marked.

7. Addition of inosine to blood immediately after collection maintains the B/2 concentration at a constant level during a two month storage period in a plastic bag. Addition of inosine to blood stored in a plastic bag for 21 days causes an appreciable rise in B/2 concentration.

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ALVEOLAR GAS TENSIONS, PULMONARY VENTILATION AND BLOOD pH DURING PHYSIOLOGIC SLEEP IN NORMAL SUBJECTS [1]

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An increase in alveolar CO_2 tension has been reported to accompany normal sleep (1, 2). The study of gas tensions during sleep has, however, been limited by two factors:

1) No accurate method of breath-to-breath analysis has been available, so that only spot checks of alveolar CO_2 tensions have been used to compare waking with sleeping levels.

2) Procedures for obtaining alveolar or arterial samples have been disturbing to the subjects. It has not been possible, therefore, to study deep sleep by these methods.

The use of a rapid acting infrared CO_2 spectrophotometer has circumvented both of these limitations and permitted an accurate and continuous study of the alveolar CO_2 changes that occur during sleep.

This paper will deal with the magnitude and mechanism of CO_2 retention during sleep, and with the changes that occur in pulmonary ventilation, respiratory gas exchange, mechanics of breathing and blood pH.

METHODS

Each subject reported to the laboratory late in the evening without special preparation. A comfortable bed in a dark environment was provided. The necessary manipulations for a control series of measurements (see below) were carried out. The subject was then allowed to fall asleep. No sedative drugs were used. All observations were made with the subject in approximately the same body position. During the course of each study, the depth of sleep as judged by degree of consciousness, amount of motion and response to stimuli was frequently recorded.

A tight-fitting face mask was used for the administration and collection of gas. The mask was attached to a low resistance valve with a small dead space. The arrangement was comfortable enough so that most subjects were able to sleep with the mask in place. During collection periods the system was frequently checked for leaks.

Expired air was collected in Douglas bags and the volume measured in a Tissot spirometer.

Alveolar CO_2 tensions were measured with a rapid acting infrared CO_2 analyzer. It has been shown by Collier, Affeldt and Farr (3) and verified by our group that in subjects without pulmonary disease, the peak end tidal CO_2 tension measured by this apparatus is equal (plus or minus 2 mm. Hg) to arterial CO_2 tension. This technique provided a breath-to-breath measurement of alveolar CO_2 tension without distraction or discomfort to the subject. CO_2 concentrations in expired air were measured by the same apparatus.

The oxygen concentrations in expired air samples were measured with a Pauling oxygen analyzer.

The volume of alveolar ventilation was calculated by the Bohr equation (4). Oxygen consumption, CO_2 production, alveolar oxygen tension, and the expiratory exchange ratio were calculated by standard methods (5, 6).

The ventilatory response to CO_2 was tested by the administration of CO_2 mixtures. Each mixture was administered for at least fifteen minutes before expired air was collected. The response to CO_2 was expressed as the ratio of the increment in alveolar ventilation to the increment in alveolar CO_2.

Appropriate vessels were intubated with No. 18 polyvinyl catheters for the collection of blood. The catheter was filled with sodium heparin solution diluted 1 to 10 with normal saline. Between periods of blood withdrawal, a sterile cap was placed on the catheter. In this manner blood could be obtained without disturbing or waking the subjects.

Blood pH was measured by means of a Beckman pH meter. Readings were made at room temperature and converted to body temperature by the Rosenthal correction factor (7).

The oxygen content and saturation of arterial blood were determined by the technique of Van Slyke and Neill (8).

The mechanics of breathing during the waking and sleeping states were studied by measurements of pulmonary compliance and mean airway resistance.[2] These

[1] This investigation was supported in part by a research grant (H-2243) from the National Heart Institute of the National Institutes of Health, Public Health Service, and in part by a grant from the Massachusetts Heart Association.

[2] These measurements were made by Dr. Roe E. Wells.

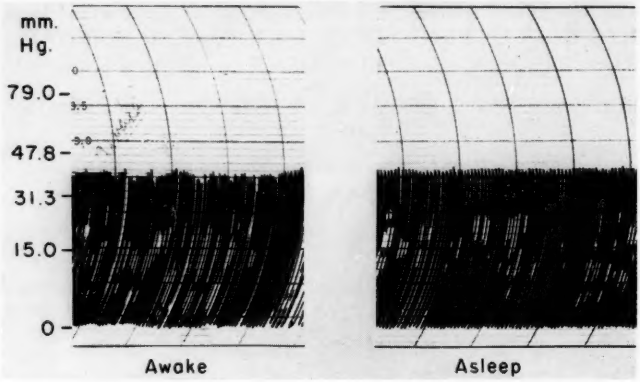

FIG. 1. A COMPARISON OF THE BREATH-TO-BREATH VARIATION OF ALVEOLAR CARBON DIOXIDE TENSION DURING THE WAKING AND SLEEPING STATES

measurements were made by the technique of Mead and Whittenberger (9). An intraesophageal balloon was used for the measurement of intrathoracic pressure. These studies were performed with the subject in the right lateral decubitus position awake and asleep.

Using these methods, breath-to-breath alveolar CO_2 tensions were obtained before and during sleep in 14 normal males. For comparison, 6 normal males were kept fully awake during the night and hourly alveolar CO_2 tensions were determined.

The ventilatory response to CO_2, awake and asleep, was tested in 10 subjects.

In three subjects arterial blood pH was determined and in two of these subjects venous pH was determined simultaneously. A consistent difference between arterial and venous pH was observed in the sixteen samples studied in this way. Therefore, the pH studies made on four additional subjects were carried out on venous blood only to obviate the necessity of an indwelling arterial catheter. Arterial oxygen saturations were determined in two subjects.

Pulmonary compliance and mean airway resistance studies were carried out in three subjects.

TABLE I

A comparison of alveolar carbon dioxide tension awake and one hour after the onset of sleep

Subject	Alveolar carbon dioxide tension *mm. Hg*	
	Awake	Asleep
R. W.	41	44
A. G.	37	41
J. M.	41	47
J. S.	38	40
J. A.	42	46
R. C.	43	45
R. W.	40	41
R. R.	40	45
L. S.	42	45
O. B.	43	47
E. R.	44	49
Mean	41.0	44.6
	$p < 0.001$	

RESULTS

The changes that occurred with sleep may be considered under the following categories:

1) The breath-to-breath *variation* of alveolar CO_2.
2) The *level* of alveolar CO_2 tension.
3) *Alveolar oxygen tension* and *arterial oxygen saturation.*
4) *Total ventilation, alveolar ventilation, oxygen consumption, CO_2 production* and *respiratory exchange ratio.*
5) *Ventilatory response* to CO_2.
6) *Blood pH.*
7) *Mechanics* of breathing.
8) *Rhythm* of respiration.

Changes in breath-to-breath variation of alveolar CO_2 tension

An analysis of variance (10) was performed on the breath-to-breath variation of alveolar CO_2 tension in four representative subjects comparing a two minute period during the waking state with a similar period during the sleeping state.

During the waking state there was a mean breath-to-breath variation of alveolar CO_2 tension of 0.8 mm. Hg. Such variation reflects changes in both metabolism and in the control of ventilation.

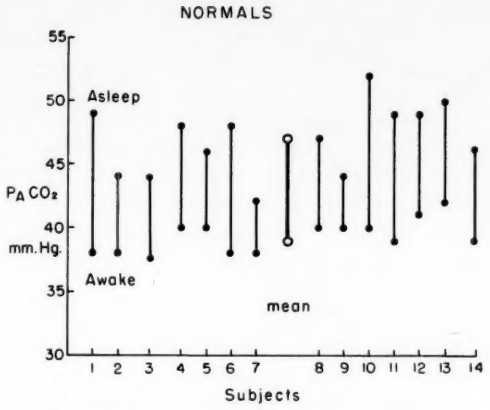

FIG. 2. PEAK ALVEOLAR CARBON DIOXIDE TENSIONS DURING SLEEP

During sleep these fluctuations virtually vanished. The mean breath-to-breath variation fell to 0.2 mm. Hg. An example of this stabilization of respiration is shown in Figure 1. The peak end tidal value of CO_2 during each breath is equal to alveolar CO_2 tension. The stability of alveolar CO_2 tension during sleep is obvious. The appearance of such stable respiration is a characteristic change that occurs with the onset of sleep. Its time of appearance correlates well with electroencephalographic changes indicating sleep (11).

Changes in the level of alveolar CO_2 tension

The infrared CO_2 analyzer provided about 7,000 measurements of alveolar CO_2 tension during each study. It is obviously not practical to report the complete data from each subject. To define the magnitude of the changes in alveolar CO_2 tension during sleep, three selected groups of data are presented:

a) A comparison of the average waking alveolar CO_2 tension with the average alveolar CO_2 tension after one hour of sleep. These data were obtained by averaging the breath-to-breath values of alveolar CO_2 tension during a two minute period while each subject was awake, and during a two minute period after the subject had been asleep for one hour. These data are shown in Table I. The mean increase in CO_2 tension after one hour of sleep is 3.6 mm. Hg. This is a highly significant change.

b) A comparison of the average waking alveolar CO_2 tension with the highest alveolar CO_2 tension attained and maintained for at least four consecutive breaths during sleep. The peak value obtained from four consecutive breaths insured that falsely high values produced by breath-holding would not be included. These data are shown in Figure 2, and it can be seen that the peak increase may be as high as 14 mm. Hg (Subject 10). The mean peak increase for the group is 9 mm. Hg which is a highly significant change.

c) Data illustrating the pattern of CO_2 increase during several hours of sleep. These data, obtained from three normal subjects, are shown in Figure 3. Alveolar CO_2 tensions are plotted along the ordinate and time in hours, along the abscissa. The straight lines connecting points indicate that continual CO_2 analysis was performed but only hourly measurements were charted. These data show considerable variation, apparently changing with the level of consciousness of the subject, but the values are higher during sleep than during waking. The peak rise in alveolar CO_2 tension tended to develop within the first one to two hours of sleep. This is consistent with the general belief that this period represents the time of deepest sleep (2).

These data from sleeping subjects may be com-

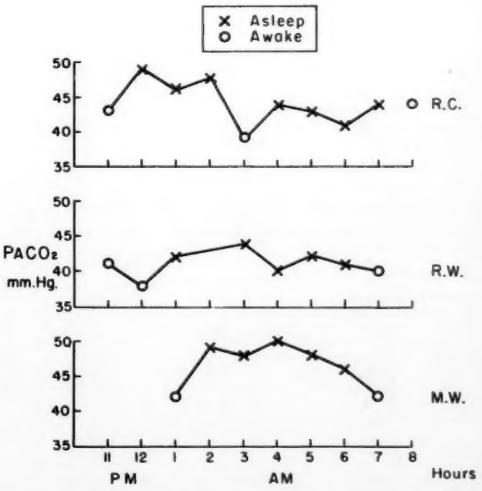

FIG. 3. HOURLY CHANGES IN ALVEOLAR CARBON DIOXIDE TENSION IN THREE NORMAL SUBJECTS

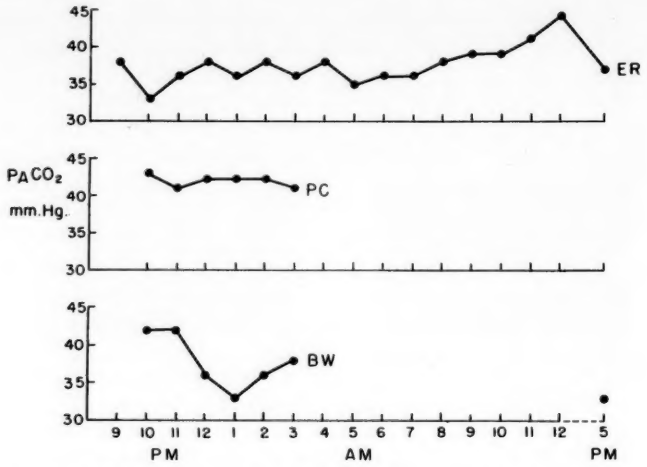

FIG. 4. HOURLY CHANGES IN ALVEOLAR CARBON DIOXIDE TENSION IN WAKING NORMALS AT NIGHT

pared with those shown in Figure 4. These data are taken from three normal subjects, awake and fully conscious during a given night of study. It can be seen that alveolar CO_2 tension in the non-sleeping subjects does not increase consistently during the night. This finding indicates that there is no normal diurnal cycle of alveolar CO_2 tension independent of sleep.

Changes in alveolar oxygen tension and arterial oxygen saturation

The data in Table II demonstrate a mean decrease in alveolar oxygen tension of 3 mm. Hg during sleep. This decrease is not statistically significant.[3] The arterial oxygen saturations during sleep, shown in Table III, confirm the absence of arterial unsaturation during sleep.

TABLE II

A comparison of alveolar oxygen tension awake and asleep

Subject	Alveolar oxygen tension *mm. Hg*	
	Awake	Asleep
A. G.	101	106
J. S.	109	109
J. A.	107	104
R. C.	106	106
R. W.	113	111
R. R.	122	111
L. S.	105	100
O. B.	105	92
Mean	108.4	104.9
	$p > 0.20$	

Changes in total ventilation, alveolar ventilation, oxygen consumption, CO_2 production and respiratory exchange ratio

Changes in total and alveolar ventilation, oxygen consumption, CO_2 production and respiratory exchange ratio during sleep are shown in Table

[3] Theoretically an increase in alveolar CO_2 tension without a change in the respiratory exchange ratio will result in a fall in alveolar oxygen tension. Alveolar oxygen tensions were calculated from experimentally obtained alveolar CO_2 tensions, expired CO_2 concentration and inspired and expired oxygen concentrations. The failure to demonstrate a significant decrease in alveolar oxygen tension in the face of an increased alveolar CO_2 tension probably is related to methodological problems. Oxygen analysis was performed by means of a Pauling oxygen analyzer which has an accuracy at best of plus or minus 1 per cent. Small errors in the determination of inspired and expired oxygen concentration will produce large errors in the calculation of respiratory exchange ratio. (This problem presumably accounts for the fact that a number of the respiratory exchange ratios shown in Table IV are greater than 1.00.) Since four separate experimental observations were necessary to calculate each alveolar oxygen tension, the inherent experimental error is obviously greater than would be expected if only a single measurement was required, as in the case of alveolar CO_2 tension.

IV. There is a mean decrease of 2.15 L. per minute in total ventilation and a mean decrease of 1.66 L. per minute in alveolar ventilation. These changes are statistically significant. There is a mean decrease in oxygen consumption of 57 ml. per minute and a mean decrease in CO_2 production of 79 ml. per minute during sleep.[4] These changes are also statistically significant. The respiratory exchange ratio is not significantly different during sleep.

Changes in the ventilatory response to CO_2

The data relating to change in the ventilatory response to CO_2 are shown in Table V. During sleep there is a mean decrease of 1.05 L. per minute of alveolar ventilation per mm. Hg of alveolar CO_2 tension. This is a highly significant decrease. The magnitude of this change is illustrated in Figure 5, where waking and sleeping response curves from three representative studies are shown.

This depression of the ventilatory response to CO_2 sets in rapidly when sleep develops and is rapidly reversed when waking occurs. This is demonstrated by the following qualitative observation. While asleep, breathing mixtures containing 4 to 6 per cent CO_2 led to only moderate augmentation of ventilation. Subjects who awoke while breathing these mixtures showed a marked hyperventilation almost *immediately* after waking. Subjects remaining awake under these circumstances continued to hyperventilate profoundly. Subjects drifting back to sleep showed a subsidence of total ventilation to the previous sleeping level.

[4] CO_2 production was measured in all 14 experimental subjects. Oxygen consumption was measured in 8 subjects.

TABLE III

A comparison of arterial oxygen saturation awake and asleep in two normal subjects

Subject	Arterial oxygen saturation %	
	Awake	Asleep
R. R.	94	94
L. S.	96	95

Changes in blood pH

The changes in pH during sleep were measured by taking samples of blood each hour since no method of continuous measurement of blood pH in humans is available. The mean value for waking and sleeping pH in each subject was obtained by averaging the values found during each state. These data are shown in Table VI and show a mean decrease of 0.03 pH unit during sleep, a statistically significant change.

Data illustrating the pattern of pH changes during sleep are shown in Figure 6. Blood pH is

TABLE IV

A comparison of total ventilation, alveolar ventilation, oxygen consumption, carbon dioxide production and respiratory exchange ratio during the waking and sleeping states

Subject	Total ventilation		Alveolar ventilation		O_2 consumption		CO_2 production		Respiratory exchange ratio	
	Awake	Asleep	Awake	Asleep	Awake	Asleep	Awake	Asleep	Awake	Asleep
	L./min. B.T.P.S.		*L./min. B.T.P.S.*		*ml./min. S.T.P.D.*		*ml./min. S.T.P.D.*			
R. W.	7.69	5.45	5.08	2.72			233	146		
A. G.	6.20	4.20	3.68	2.14	232	120	154	99	0.66	0.82
J. M.	8.64	5.17	6.42	3.33			399	174		
J. S.	7.99	4.97	4.80	3.30	204	163	204	157	1.00	0.96
J. A.	6.31	6.04	3.72	3.87	190	197	174	197	0.92	1.00
R. C.	8.10	6.06	5.09	3.76	258	202	242	198	0.94	0.98
R. W.	11.60	7.13	7.47	4.17	303	182	337	193	1.04	1.06
R. R.	6.23	4.80	4.47	2.91	183	122	192	144	1.05	1.18
L. S.	7.72	8.41	5.63	5.29	256	300	246	279	0.96	0.93
O. B.	9.89	8.32	6.67	5.30	342	351	359	276	1.05	0.79
E. R.	6.12	3.87	4.25	2.28			244	147		
C. C.	8.65	5.41			319	202	311	184	0.97	0.91
R. W.	7.99	5.32			314	192	296	176	0.94	0.92
Mean	7.93	5.78	5.21	3.55	260	203	261	182	0.95	0.96
p	<0.001		<0.001		<0.025>0.01		<0.05>0.005		not sig.	

TABLE V

A comparison of the ventilatory response to carbon dioxide during the waking and sleeping states

Subject	Δ Alveolar ventilation	
	Awake	Asleep
	L./min./mm. Hg CO_2 tension	
R. W.	3.30	0.13
A. G.	1.32	0.57
J. M.	2.03	0.43
J. S.	0.65	0.22
J. A.	0.94	0.46
R. C.	1.09	0.72
R. R.	0.79	0.45
L. S.	1.30	0.33
O. B.	0.75	0.04
E. R.	1.88	0.12
Mean	1.40	0.35
p	<0.001	

TABLE VI

A comparison of blood pH during the waking and sleeping states

Subject	Source	Average blood pH	
		Awake	Asleep
L. T.	Artery	7.43	7.42
M. W.	Artery	7.40	7.37
M. P.	Artery	7.45	7.41
P. N.	Vein	7.44	7.41
T. A.	Vein	7.46	7.41
M. P.	Vein	7.41	7.37
R. R.	Vein	7.42	7.40
Mean		7.43	7.40
	p<0.001		

plotted along the ordinate and time in hours, along the abscissa. It can be seen that blood pH tends to fall during sleep. The decrease in pH, however, is not present as consistently as is the rise in alveolar CO_2 tension.

Mechanics of breathing

The effect of sleep on pulmonary compliance and mean airway resistance is shown in Table VII. There are small and probably insignificant changes in pulmonary compliance while airway resistance showed a small but consistent decrease during sleep. The number of subjects studied is not large enough to permit statistical analysis for significance. However, these data indicate that sleep does not produce an *increase* in airway resistance or a *decrease* in pulmonary compliance. Thus the work of breathing, if anything, is less during sleep than in the waking state.

Rhythm of respiration

Six of 14 *normal* subjects showed cyclic breathing of the Cheyne-Stokes variety during sleep. These variations in the rhythm of breathing differed from true Cheyne-Stokes breathing in that

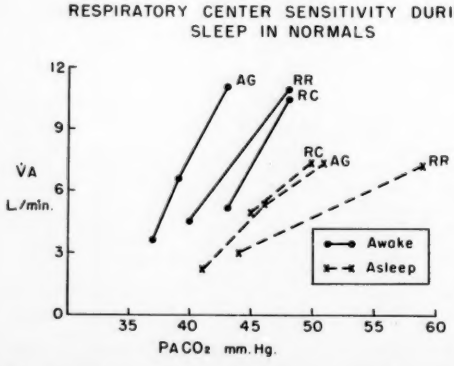

FIG. 5. WAKING AND SLEEPING VENTILATORY RESPONSE TO CARBON DIOXIDE IN THREE NORMAL SUBJECTS

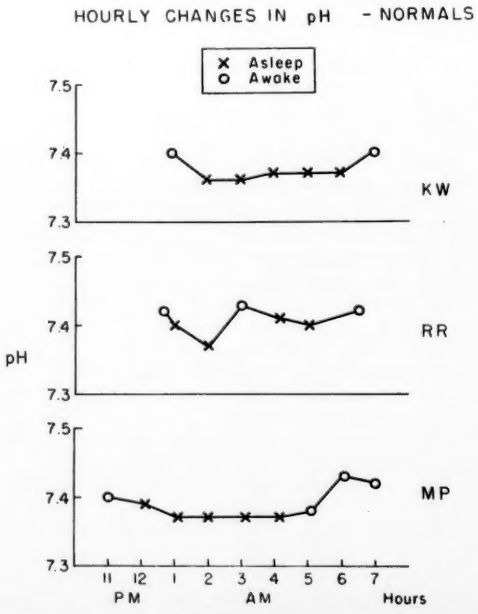

FIG. 6. HOURLY CHANGES IN BLOOD pH IN THREE NORMAL SUBJECTS

periods of hyperventilation alternated with hypopnea rather than with apnea. In normal subjects periodic breathing was only found transiently. No subject demonstrated this variety of breathing longer than one hour during a night of sleep. An example of this phenomenon is shown in Figure 7.

DISCUSSION

The increase in alveolar CO_2 tension during sleep is a consistent finding. Alveolar CO_2 tension may be increased by either of two mechanisms:

1) An increase in CO_2 production.

2) A decrease in alveolar ventilation.

Since the data show that sleep is accompanied by a decrease rather than an increase in CO_2 production, the CO_2 retention of sleep is to be explained by a decrease in alveolar ventilation. This decrease in alveolar ventilation is apparently related to a depression of respiratory center sensitivity to CO_2. This is indicated by the marked decrease in the ventilatory response to CO_2 observed during sleep.

The ventilatory response to CO_2 may be accepted, with some reservations, as a quantitative expression of respiratory center sensitivity to CO_2 (12, 13). The reservations are as follows:

1) Inhaled CO_2 stimulates the peripheral chemoreceptors in the aortic and carotid bodies as well as the medullary chemoreceptors (14).

TABLE VII

Mechanics of breathing during sleep in three normal subjects

Subject	Pulmonary compliance		Mean airway resistance	
	Awake	Asleep	Awake	Asleep
	L./cm. H_2O		*cm. H_2O/L./sec.*	
E. R.	0.238	0.179	2.18	1.48
D. J.	0.164	0.203	2.03	1.09
D. T.	0.177	0.201	2.40	1.42

2) The CO_2 tension and pH of arterial blood and of the respiratory center are related but are not necessarily equal. At present, techniques for the measurement of intracellular pH and CO_2 are not available.

3) Any factor which modifies the volume of ventilation may modify the ventilatory response to CO_2. It has been shown by Cherniack and Snidal (15) in normal subjects that the ventilatory response to CO_2 may be depressed by increasing airway resistance. Fritts, Fishman and Cournand (16) have suggested that the depressed ventilatory response to CO_2 in emphysema may be related to the increased work of breathing in this disorder.

Since pulmonary compliance is not decreased and mean airway resistance is not increased by sleep, it appears that the depression of the ventilatory response to CO_2 during sleep is not related to impairment of the mechanics of breathing.

It therefore seems probable that the changes in

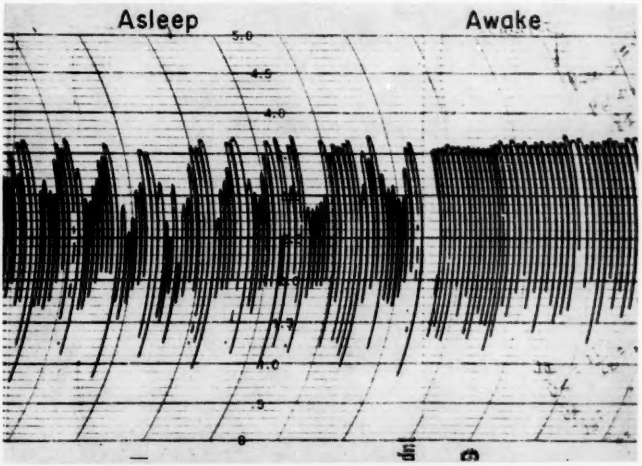

FIG. 7. PERIODIC BREATHING IN A NORMAL SUBJECT DURING SLEEP

the ventilatory response to CO_2 during sleep represent changes in respiratory center sensitivity to CO_2. Since these changes take place within seconds, it seems probable that they are mediated neurogenically.

A working hypothesis to explain the respiratory acidosis of sleep may be stated as follows: The sensitivity of the respiratory center to CO_2 is dependent on, among other factors, afferent impulses coming to it from numerous areas in the nervous system. During the waking state the number of afferent impulses is relatively large, producing a brisk alveolar ventilation and resulting in a normal waking alveolar CO_2 tension. During sleep, the number of impulses is decreased and the responsiveness of the respiratory center consequently decreases. The result is a decreased level of alveolar ventilation and a rise in alveolar CO_2 tension until a new steady state is achieved.

The elevated alveolar CO_2 tension during sleep reported here is in agreement with the findings of most previous studies (1, 2). Mills has reported that the elevation of alveolar CO_2 tension that takes place during the night occurs as a result of a normal diurnal cycle and is independent of whether or not the subject is sleeping (17). Our finding that normal subjects kept awake at night show no respiratory acidosis differs from the results reported by Mills. It should be pointed out that Mills studied only one subject (himself) during sleep. In addition, his technique of study consisted of utilizing a Haldane tube for the determination of alveolar CO_2 tensions. At various times during the night an alarm clock roused him from sleep so that a sample of expired air might be obtained. In view of the rapid changes in CO_2 tensions that we have observed in the transition from the sleeping to the waking state, it is doubtful that Mills' observations can be considered as representing alveolar CO_2 tensions *during sleep*.

Changes in the cerebral circulation have been investigated by workers in the field of sleep. It seems probable that the changes observed may be related to changes in alveolar gas tensions. Thus, the increase in cerebral blood flow and decrease in cerebral vascular resistance during sleep reported by Mangold and co-workers (18) may be accounted for by the increase in alveolar CO_2 tension.

Doust and Schneider (19) have proposed a theory which ascribes sleep to cerebral hypoxia. Using the technique of ear oximetry these authors found that arterial oxygen saturation decreased progressively during the process of sleep, reaching its lowest levels during deep sleep.

In our own studies a mean decrease of 3 mm. Hg in alveolar oxygen tension was found during sleep, but this decrease was not statistically significant. The shape of the oxyhemoglobin dissociation curve is such that the saturation values for highly saturated blood fall on the flat portion of the curve. Under these circumstances, even subjects who showed the greatest decrease in alveolar oxygen tension did not have a decrease of sufficient magnitude to produce arterial oxygen unsaturation. In the subject whose hemoglobin is unsaturated in the waking state the findings may be different. Because of the shape of the oxyhemoglobin dissociation curve a small decrease in alveolar oxygen tension may produce a significant decrease in arterial oxygen saturation.

At any rate, no evidence of significant arterial oxygen unsaturation was found in our normal subjects during sleep, and thus the hypoxic theory of the genesis of sleep appears to be untenable. The possibility of a marked decrease in oxygen saturation during sleep in subjects who are moderately unsaturated while awake is under investigation.

The data reported here do not in any sense define the fundamental nature of the sleeping state. They lend themselves, however, to several generalizations:

1) The basic changes associated with sleep are rapidly reversible. The rapidity of these changes suggests that neurogenic mechanisms play a decisive role in the genesis and maintenance of normal sleep.

2) Sleep does not represent a static state. The sleeping subject shows changes in the depth of sleep, the number of bodily movements (2), alveolar gas tensions, electroencephalographic patterns (11) and blood pH.

3) Theories of the genesis of sleep based on changes in blood gas constituents are probably untenable.

SUMMARY

Physiologic sleep in the normal subject is associated with a significant fall in total and alveolar

ventilation, with a significant rise in alveolar CO_2 tension, with a fall in CO_2 production and oxygen consumption, with a fall in ventilatory response to CO_2, with a fall in blood pH, and with a considerable incidence of periodic breathing. Alveolar oxygen tension, arterial oxygen saturation, respiratory exchange ratio, and the mechanics of breathing are not significantly altered by sleep.

The CO_2 retention of sleep is related to a depression of respiratory center sensitivity. The speed of onset and reversal of this depression of respiratory center sensitivity suggests that it is mediated neurogenically.

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BLOOD AMMONIA LEVELS FOLLOWING THE INGESTION OF CASEIN AND WHOLE BLOOD [1]

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In the recent past, much attention has been focused on the role of ammonia in hepatic coma. A relation between gastrointestinal bleeding and the precipitation of hepatic coma has been noted (1, 2). Various substances have been administered into the gastrointestinal tract, such as urea, ammonium chloride, exchange resins of the ammonia cycle, high protein feedings, and blood (3–7), all of which can produce elevated blood ammonia levels and precipitate hepatic coma in susceptible individuals. The implicit assumption, if not explicit expression, in these various studies, has been that all of these substances are more or less equally noxious agents in regard to elevating blood ammonia and causing the concomitant neurological changes. The problem of irreversible coma in patients with liver disease following gastrointestinal bleeding is frequently found; yet high protein diets are standard fare in the treatment of cirrhosis, and relatively infrequent catastrophies result. We have compared the relative effect of the ingestion of protein derived from blood as compared to protein derived from casein and milk solids (Protenum®) on the blood ammonia and nonprotein nitrogen (NPN).

METHODS

Observations were made on a control group and a group of patients with liver disease. The control group consisted of three patients with cerebrovascular accidents and generalized arteriosclerosis but no demonstrable liver disease, and one patient with anxiety neurosis and no demonstrable liver disease. Of the patients with liver disease, six had Laennec's cirrhosis and one had diffuse carcinomatosis with hepatic involvement.

In the control group two patients were given both blood and Protenum® at different times. In these patients the total amount of protein nitrogen administered in the blood and Protenum® was the same. A third control patient received only blood and the fourth control patient received only Protenum®. All patients with liver disease received both blood and Protenum®, at suitable intervals, at times when they were considered to be relatively stable in regard to their liver disease. Four patients received Protenum® first and three patients received the blood first. F. G., R. V., E. B. and C. D. ingested Protenum® first.

The blood used for intragastric administration was blood collected under standard blood bank conditions and kept refrigerated until used. Prior to administration aliquot specimens of the bottles of blood from the blood bank were assayed in duplicate for total nitrogen by the macro-Kjeldahl method (8). Total nitrogen in weighed samples of Protenum® was similarly measured. On the basis of these determinations suspensions of weighed amounts of Protenum® could be made equivalent in volume and nitrogen content to any given quantity of the bank blood. Protenum® is made from nonfat milk solids, calcium caseinate, dextrose, cocoa, vitamins and minerals [by analysis, the protein content ($N \times 6.38$) is 42 per cent by weight, according to the manufacturer].

All patients were in a fasting state. After initial blood specimens were taken, they received the blood or Protenum® in approximately the same amount of total fluid containing the same quanitity of total nitrogen, over a 20 to 30 minute period of time, via nasal tube or by ingestion. Arterial and venous blood samples were drawn simultaneously whenever feasible at 0, 1, 2.5, 4 and 5 hours after administration of the test substance. The patients were observed for any development of nervousness, tremors, and so forth. The blood samples were analyzed for ammonia and NPN content. A modification of the method of Seligson and Seligson (9) was used for ammonia determinations. Normal values for blood ammonia levels in our laboratory range from 0.60 to 1.10 μg. per ml. The values are expressed as μg. of ammonia nitrogen per ml. NPN's were determined by the method of Folin and Wu (10).

RESULTS

Table I presents the results of the protein analysis of the various blood specimens which are administered to the patients. As can be seen from the data there is a wide variation in the protein concentration of the various blood specimens, all of which were drawn in the usual fashion at a

[1] Supported by a grant from the United States Public Health Service, National Heart Institute.

blood bank. The highest protein content is found in the blood given Patient F. S., 20.60 Gm. per cent (Table I); the lowest is seen in that given Patient L. V., 12.31 Gm. per cent. The other values are fairly equally distributed between the two extremes. Thus it can be seen that 500 ml. of the 20.60 Gm. per cent blood would contain 103 Gm. of protein, while an equivalent quantity of the blood given Patient L. V. would contain only approximately 62 Gm.—a 40 per cent difference.

In Table II are presented the clinical diagnoses on the various patients and some of the criteria employed to evaluate the presence and extent of liver disease. The patients with cirrhosis were studied at a time during which it was felt that their liver disease was quiescent and stable. R. S., the patient with carcinomatosis, showed a slow but steady downhill course during the study. In this case the blood was given first. Therefore, if there were any difference in liver function between the two studies it would have been slightly better when the blood was given than when the Protenum® was given. Patients with viral hepatitis or other acute liver disease were excluded from this study since it was felt that their liver disease would be in such a labile state that two studies, even only a few days apart, would not be comparable.

TABLE I

Protein content of blood administered to patients

Patient	Protein
	Gm. %
A. Patients with no liver disease	
A. R.	12.50
M. K.	19.20
W. L.	18.50
B. Patients with liver disease	
F. S.	20.60*
F. G.	19.00
L. V.	12.31†
R. S.	16.10
R. V.	16.56
E. B.	13.75
C. D.	17.50

* Highest protein content.
† Lowest protein content.

Table III presents the levels of arterial and venous blood ammonia over a five hour period following the intragastric administration of approximately 500 ml. of whole bank blood, given in a period of 15 to 20 minutes. It can be seen (III, A) that the fasting blood ammonia of control patients ranged very closely around 1 μg. per ml. The arteriovenous differences were very close to zero. The maximum rise of the blood ammonia level was

TABLE II

Liver function studies

Patient	Diagnosis	Ascites	Hepato-megaly	Cephalin floccu-lation	Thymol	Bili-rubin	Brom-sulph-alein	Total protein	Albumin/ globulin	Hema-tocrit	Biopsy
							%				
A. R.	Cerebral vascular accident	0	0	1+	0.25	<1.0		5.8	3.6/2.2	36	
M. K.	Cerebral vascular accident	0	0	2+	0.80			6.6	3.9/2.7	47	
W. L.	Cerebral vascular accident	0	0	1+	0.50	<1.0				41	
R. Mc.	Anxiety neurosis	0	0	1+	1.0		<5			43	
Liver disease											
F. S.	Cirrhosis	+++	3+	3+	4.0	2.7		7.9	3.5/4.4		+
F. G.	Cirrhosis; pul. tbc.	0	4+	2+	1.0	3.4		6.9	2.6/4.3	30	+
L. V.	Cirrhosis	0	2+	4+		7.3		7.4	1.9/5.5	42	
R. S.	Carcinomatosis with liver involve.	0	4+	4+	1.0	2.5		5.9	3.2/2.7	26	+
R. V.	Cirrhosis	+	3+	4+	3.7	4.3		7.1	3.3/3.8	31	+
E. B.	Cirrhosis	0	2+	1+	2.3	3.0	12.5	6.4	3.9/2.5	42	
C. D.	Cirrhosis	0	2+	1+	2.0	1.5	10.0	6.1	3.6/2.5	43	+

TABLE III

Ammonia levels following whole blood administration (μg. per ml.)

Patient	Protein received	Fasting			1 hour			2.5 hours			4 hours			5 hours		
		A	V	A-V	A	V	A-V	A	V	A-V	A	V	A-V	A	V	A-V
	Gm.															
A. Patients with no liver disease																
A. R.	63	1.03	1.03	0.00	1.28	1.06	+0.22	1.07	1.00	+0.07	1.10	1.30	−0.20	1.10	0.89	+0.21
M. K.	86	0.85	0.79	+0.06	0.92	1.10	−0.18	0.96	1.06	−0.10	0.82	1.10	−0.28	1.16	1.04	+0.12
W. L.	91	0.76	0.69	+0.07	0.86	1.34	−0.48	1.20	1.38	−0.18	0.75	0.95	−0.20	0.88	1.00	−0.12
Average	80	0.88	0.83	+0.04	1.02	1.17	−0.15	1.08	1.15	−0.07	0.89	1.12	−0.23	1.05	0.98	+0.07
B. Patients with liver disease																
F. S.	103	2.00	1.54	+0.46	2.52	1.80	+0.72	3.01	2.20	+0.81	3.00	2.48	+0.52		1.65	
F. G.	104	2.09	1.74	+0.35	3.52	2.46	+1.06	4.60	4.12	+0.48	4.48	4.28	+0.20	4.72	4.43	+0.29
L. V.	74	1.38	1.33	+0.05	2.30	1.76	+0.54	4.00	3.36	+0.64	3.95	2.77	+1.8	3.00	2.85	+0.15
R. S.	80	2.00	1.56	+0.44	2.32	1.98	+0.34	2.83	2.93	−0.10	3.54	3.62	−0.08	3.34	3.22	+0.12
R. V.	75	1.94			2.60	2.30	+0.30	4.06	3.38	+0.68	3.92	3.76	+0.16	3.35	3.25	+0.10
E. B.	80	1.48	1.36	+0.12	2.00	1.60	+0.40	2.68	1.90	+0.78						
C. D.	70	1.16	1.22	−0.06	1.93	1.46	+0.47	2.36	1.65	+0.71				1.84	1.69	+0.15
Average	84	1.72	1.46	+0.23	2.45	1.92	+0.53	3.36	2.79	+0.57	3.78	3.38	+0.40	3.25	2.85	+0.16

seen in Patient W. L. who showed an increase of approximately 0.6 μg. per ml. in both arterial and venous values. In contrast (Table III, B), the ammonia levels of the patients with liver disease rose significantly in all instances and rather abruptly in several cases. Furthermore there was a marked delay in some patients before the levels returned toward normal. In none of the patients with liver disease had the blood ammonia levels returned to normal by five hours. The most pronounced elevation of blood ammonia was seen in Patient F. G. after 104 Gm. of protein in the form of blood was administered. At 2.5, 4 and 5 hours, his ammonia levels were about 2.5 μg. per ml. higher than the fasting level, and he exhibited symptoms of nausea, tremulousness and nervousness. Patient L. V., who showed an equivalent rise in blood ammonia, also manifested nervousness and irritability during the period of peak elevation. An interesting finding in Patient R. S. was the lack of a positive arteriovenous difference despite distinct elevation in ammonia levels. This phenomenon will be discussed later. Patients R. V. and E. B. showed more or less the same pattern as the first three patients. C. D. received the lowest total protein dose and clinically had rather mild cirrhosis. Nevertheless, his peak ammonia level was 1.2 μg. higher than the fasting

TABLE IV

Ammonia levels following Protenum® administration (μg. per ml.)

Patient	Protein received	Fasting			1 hour			2.5 hours			4 hours			5 hours		
		A	V	A-V	A	V	A-V	A	V	A-V	A	V	A-V	A	V	A-V
	Gm.															
A. Patients with no liver disease																
A. R.	70	1.33	1.11	+0.22	1.30	1.04	+0.26	1.08	1.26	−0.18	1.47	1.50	−0.03	1.14	1.21	−0.07
M. K.	86	0.69	0.72	−0.03	0.85	0.91	−0.06	0.76	0.70	+0.06	0.73	1.02	−0.29	0.94	1.00	−0.06
R. Mc.	120	0.94	0.79	+0.15	0.90	0.82	+0.08	1.04	1.01	+0.03	1.04	0.93	+0.11			
Average	90	0.98	0.87	+0.11	1.01	0.92	+0.09	0.96	0.99	−0.03	1.08	1.15	−0.07	1.04	1.10	−0.06
B. Patients with liver disease																
F. S.	91		1.42		2.34	1.94	+0.62	2.43	1.94	+0.49		1.61			1.58	
F. G.	120	1.78	1.40	+0.38	3.35	2.13	+1.22	2.49	2.33	+0.16	1.92	1.71	+0.21	2.00	1.58	+0.42
L. V.	74	1.82	1.75	+0.07	1.82	1.64	+0.18	2.09	1.90	+0.19	1.81	1.65	+0.16	1.69		
R. S.	80	1.22	1.10	+0.12	1.20	1.16	+0.04	1.50	1.18	+0.32	1.33	1.09	+0.24	1.49	1.21	+0.28
R. V.	75	1.53	1.13	+0.40	2.14	1.23	+0.91	1.69	1.33	+0.36	1.60	1.13	+0.47			
E. B.	80	1.12	0.92	+0.20	1.24	1.26	−0.02	1.21	1.24	−0.03						
C. D.	70	1.20	1.00	+0.20	1.36	1.26	+0.10	1.34	1.06	+0.28				1.53	1.26	+0.27
Average	84	1.44	1.24	+0.23	1.92	1.48	+0.44	1.82	1.57	+0.25	1.66	1.44	+0.27	1.67	1.41	+0.32

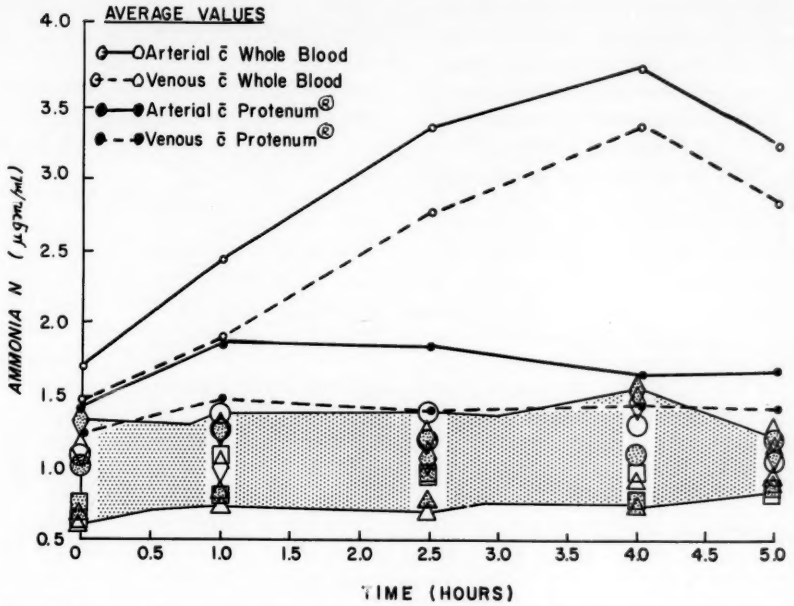

FIG. 1. AVERAGE BLOOD AMMONIA LEVELS (SEVEN PATIENTS) FOLLOWING ADMINISTRATION OF BLOOD AND PROTENUM®

Shaded area represents maximum and minimum arterial and venous blood ammonia levels of control patients similarly treated.

Stippled and open circles—maximum arterial and venous levels after blood administration; stippled and open squares—minimum arterial and venous levels after blood administration; stippled and open diamonds—maximum arterial and venous levels after Protenum® administration; stippled and open triangles—minimum arterial and venous levels after Protenum® administration.

level, an increment twice as great as the highest seen in the control group. None of the patients except F. G. and L. V. showed central nervous system symptoms during the test. It can be noted that all patients with liver disease, with the exception of R. S., showed a positive arteriovenous difference of ammonia, and this difference usually increased parallel with the elevation of the arterial ammonia level.

Table IV gives the levels of blood ammonia in patients without liver disease (A) and with liver disease (B) following the intragastric administration of Protenum®. A. R. was given slightly more protein in the Protenum® administration than in the blood administration; M. K. was given the same amount. Two other patients without liver disease were studied. W. L. was given blood containing 91 Gm. of protein (see Table III), and R. Mc. was given 120 Gm. of protein in the form of Protenum®. There were no marked changes in the blood ammonia levels in any of the normal subjects. All of the patients with liver disease received the same total amount of protein as they had received from the blood, with the exception of the first two patients. The first patient, F. S., received 12 Gm. more protein from the blood source, and F. G. received 16 Gm. more protein as Protenum®. It is of interest to note that in this latter patient (F. G.), despite the greater amount of protein received from Protenum®, the elevation of blood ammonia levels was far less and of shorter duration than when the blood was given. This same pattern is repeated in all of the patients; that is, for an equivalent amount of protein administered the increase in the levels of blood ammonia was much less marked when Protenum®

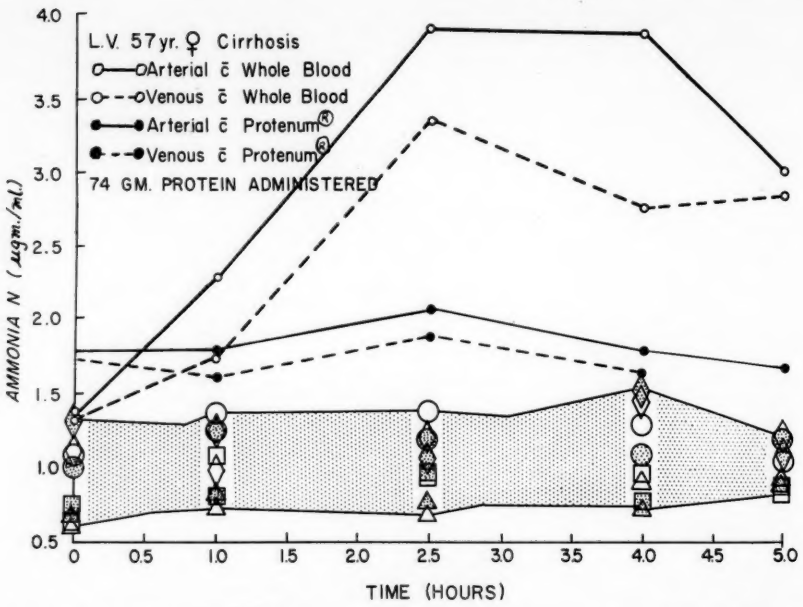

FIG. 2. BLOOD AMMONIA LEVELS OF TYPICAL PATIENT WITH CIRRHOSIS AFTER ADMINISTRATION OF BLOOD AND PROTENUM®

Shaded area represents maximum and minimum arterial and venous blood ammonia levels

was given than when blood was given. None of the patients demonstrated any signs or symptoms of disturbance of central nervous system following Protenum® administration. In some patients the changes in the levels of blood ammonia were minimal.

Figure 1 illustrates in graphic form the average arterial and venous levels of ammonia over the five hour test period for the patients with liver disease following both Protenum® and blood administration. The shaded area across the bottom represents maximum and minimum levels of the

TABLE V

Nonprotein nitrogen (NPN) levels following blood and Protenum® administration (mg. per cent)

Patient	Protein received (Gm.)		Fasting		1 hour		2.5 hours		4 hours		5 hours	
	Blood	Protenum®	Blood	Protenum®	Blood	Protenum®	Blood	Protenum®	Blood	Protenum®	Blood	Protenum®
A. Patients with no liver disease												
A. R.	63	70		18.52		22.72		23.10		20.05		18.97
M. K.	86	86	58.50	33.22	49.50	29.77	52.50	34.05	54.00	33.22	54.00	
R. Mc.		120		28.20		33.27		29.85		34.42		
B. Patients with liver disease												
F. S.	103	91	19.72	20.02	23.47	22.57	32.10	20.02	26.40	17.02	23.47	36.15
F. G.	104	120	12.45	13.37	19.95	17.65	22.05	18.75	22.87	17.65	10.35	18.30
L. V.	74	74	20.77	21.22	24.52	19.95	32.85	21.22	44.47	9.50	29.55	23.32
R. S.	80	80	20.77	33.30	38.70	33.75	39.52	33.30	47.47	35.40	52.05	41.25
R. V.	75	75	15.00	21.22	19.72	25.35	22.50	24.15	27.60	24.15	25.20	
E. B.	80	80	17.20	17.90	25.80	19.51	29.70	20.83				
C. D.	70	70	21.60	25.45	36.22	28.28	35.40	25.04			34.60	32.38
Average	84	84	18.21	21.78	26.91	23.86	30.58	23.33	33.16	20.74	29.20	25.23

normals. Figure 2 shows the results of one typical patient, L. V., with the normal range again shaded in.

Table V gives the levels of NPN on the same patients. The rise in NPN's tended to parallel the changes in blood ammonia.

DISCUSSION

The patients chosen for evaluation in the study were known cirrhotics (six out of seven) of fairly long standing, neither convalescing from an acute episode nor noticeably deteriorating. In a group of patients who are thought to have clinically equivalent liver disease the liver function test may differ markedly from patient to patient. Conversely, although biochemically the same, two cirrhotic patients may appear very different clinically. Therefore it was felt that comparison of one group of patients with another group of patients would not yield as valid or worthwhile information as would the comparison of each patient with himself at relatively short intervals.

The total amount of protein administered in the form of blood and Protenum® was the same for both test substances (with the exceptions noted above) for a given subject. However, all subjects did not receive the same total amount of protein. An attempt was made to vary the dose of total protein from patient to patient according to an estimate of the severity of each individual's liver disease in order to avoid precipitating hepatic coma. Despite this variation in dosage, all patients showed the same pattern of response, *i.e.*, all had a greater rise in blood ammonia after ingestion of blood than after Protenum® ingestion.

The average ammonia level of the bank blood administered was 10 μg. per ml. If a total of 500 ml. of blood was given (and this was the average amount administered), the total ammonia given would be 5 mg., roughly equivalent to 20 mg. of ammonium chloride. This amount of ammonia distributed unaltered throughout the body fluids would cause an increase of 0.125 μg. per ml. in a 70 kilogram individual. This is an insignificant amount even though it has been remarked upon by Spear, Sass, and Cincotti (11). We therefore feel that the free ammonia in the bank blood did not contribute to the marked elevations of the blood ammonia levels noted following ingestion of blood. The average level of ammonia in Protenum® was 6 μg. per ml. Further evidence that the ammonia in transfused blood is negligible in effect can be obtained from the data of Gorten, Shear, Hodson, and Bessman (12) concerning the use of fresh blood for exchange transfusion. Here it is shown that blood with an ammonia content of up to 3 μg. per ml. had no effect in the blood ammonia level of the recipient, even though more than ninety per cent of the patient's blood was exchanged.

An uptake of ammonia both by cerebral and peripheral tissues has been demonstrated by Bessman, Fazekas, and Bessman (13, 14). In this study a correlation between arteriovenous differences (*i.e.*, uptake of ammonia by the tissues) and the degree of elevation of the arterial ammonia was observed. In general, the greater the elevation of arterial blood ammonia, the greater the arteriovenous difference. These observations have been confirmed by some (15) and disputed by others (16). Even in this latter report, however, the data in Figure 6 appear to show a similar correlation. Six of the seven patients with liver disease in the present study showed correlation of arteriovenous difference of ammonia with a rise in the arterial levels. One patient (R. S.) showed no difference whatsoever, even on pronounced elevation of the arterial level. This patient was extremely thin and his muscle mass greatly reduced. It may be that in certain conditions such as extreme debility or cachexia, capacity of muscle for removing ammonia has already been reached and no increased activity is possible. Terminal coma patients have been observed to have a similar reversal of arteriovenous differences (17). This phenomenon is not consistent, nor is it explicable at the present time.

The deleterious effects of gastrointestinal bleeding in patients with cirrhosis have long been known. Welch, Kiley, Reeve, Goodrich, and Welch (1) reported a 76 per cent mortality in patients with cirrhosis during their first hemorrhage. Over 90 per cent of these patients had proven varices. The presence of cirrhosis in a patient with a bleeding duodenal ulcer makes for a more guarded prognosis (2). Some of these deaths are attributable, of course, to uncontrollable hemor-

rhage. However, patients with severe liver disease can be precipitated into hepatic coma by gastrointestinal bleeding. In a series recently reported by Summerskill, Wolfe, and Davidson (5), gastrointestinal hemorrhage precipitated or complicated hepatic coma in 12 of 34 patients.

Young, Burnside, Knowles, and Schiff (7) have shown that patients with liver disease given 500 ml. of bank blood show a far greater rise in blood ammonia levels than do normal controls. In this study, one pint of blood was administered without the amount of total nitrogen in that specimen having been determined. Our measurements have shown that one pint of blood may contain from 62 to 136 Gm. of protein. Other investigators have shown elevations in blood ammonia levels following administration of ammonium chloride, urea and high protein feedings (3–6, 18). These changes would seem to be of the same order of magnitude as those shown by Young and coworkers following blood administration. Furthermore, it has been reported that diets of 120 Gm. of protein may precipitate coma in patients with cirrhosis and that occasionally patients have been sensitive to as little as 20 Gm. of protein (5). Patients so sensitive to dietary protein (if it can be likened to Protenum®) would probably be inordinately sensitive to blood in the gastrointestinal tract.

The present study has shown that per Gm. of nitrogen administered, ingested blood causes a higher level of blood ammonia than protein from a casein and milk solids source. Certain amino acids such as methionine (19) have been shown to be particularly toxic in patients with liver disease, probably through their effect on blood ammonia. Others, such as glutamic acid and arginine, have been shown to have protective properties in preventing ammoniagenic coma or in ameliorating its symptoms when such symptoms are precipitated by a sudden ingestion of substances previously mentioned (blood, ammonium chloride, urea, and so forth). One of the problems now under study is whether blood contains more of the toxic amino acids or whether Protenum® contains more of the protective ones.

Certain practical therapeutic considerations follow from the above study. In the treatment of gastrointestinal hemorrhage in patients with liver disease several procedures would seem advisable in addition to the usual ones of blood replacement, treatment of shock, and so on. These studies would support the views of those who advocate removal of the blood from the gastrointestinal tract by aspiration and the use of enemas (5). The rationale of the use of antibiotics such as chlortetracycline (19) and neomycin (20) rests on the inhibition of enteric bacterial flora which increase proteolysis. Certainly the administration of one of these drugs would be advisable in the treatment of gastrointestinal hemorrhage in patients with liver disease.

The adjuvant use of sodium glutamate and/or arginine is also indicated in the treatment of ammoniagenic coma precipitated by gastrointestinal bleeding in patients with liver diseases. Various investigators have used glutamate in the therapy of hepatic coma with varying reports as to its success or failure (21–24). However, even in those instances in which the patient did not respond clinically, the blood ammonia levels usually fell during or shortly after glutamate administration. Other workers have used arginine (25, 26) to lower blood ammonia levels. In a recent study it was shown that in cirrhotic patients glutamate was more effective than arginine, mole for mole, in preventing the elevation of blood ammonia produced by administration of ammonium chloride (27).

The observation has been made that in conditions such as gastrointestinal hemorrhage, shock, and so forth, there exists a state known as prerenal azotemia, characterized, among other things, by an oliguria and an elevated NPN. These abnormal findings usually return to normal with the correction of the primary lesion. In the present study, as well as in the observations of Young and associates (7), the rise of the NPN paralleled the rise of the blood ammonia. In no case in which the blood ammonia levels remained stationary did the NPN levels increase; and conversely, in no cases in which the ammonia levels rose did the NPN levels fail to rise. Nelson and Seligson (28) have shown blood ammonia levels to be elevated in shock, and this study as well as others has shown the blood ammonia levels to be elevated by the presence of blood in the gut. Thus it may be that a part of the elevated NPN which

characterizes prerenal azotemia may be due to the presence of elevated blood ammonia, which furnishes a source for the rapid synthesis of urea, glutamine and other components of the nonprotein nitrogen.

CONCLUSIONS

1. Blood and a casein-milk solids preparation (Protenum®) were administered intragastrically to control patients and patients with liver disease. Each patient received both test substances and for a given patient the total nitrogen content of the two test substances was the same.

2. None of the control subjects showed significant elevations in either blood ammonia or the nonprotein nitrogen (NPN) over a period of three to five hours.

3. All seven of the patients with liver disease showed a significant rise in blood ammonia and NPN following administration of blood; six of these patients showed a significant rise in these determinations following the administration of Protenum®. In the patients with liver disease, ammonia and NPN levels following administration of blood were uniformly higher than following Protenum®.

4. Several patients developed central nervous system symptoms during the period of elevation of blood ammonia levels.

5. The arteriovenous difference of ammonia increased in six of the seven patients with liver disease as the arterial levels increased.

6. The practical significance of these findings in relation to the treatment of gastrointestinal hemorrhage is discussed.

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PATHWAYS OF CARBOHYDRATE FORMATION IN MAN. I. ISOTOPE DISTRIBUTION IN GLUCOSE FROM NONDIABETIC SUBJECTS GIVEN 1-C^{14}-ACETATE [1, 2]

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A number of studies with tracers in animals have been done to investigate the metabolic pathways by which carbon of fatty acid is converted to carbohydrate. Analysis of distribution of isotope in the carbon positions of glucose (or glycogen) formed *in vivo* from position-labeled precursors provides insight into the metabolic routes traversed (2, 3). Such studies have generally upheld the concept that fatty acids are degraded to two-carbon units, then incorporated into the tricarboxylic acid cycle, from which three-carbon monocarboxylic acids of the glycolytic pathway and finally hexose derivatives are formed. In conformity with this scheme are the findings that fatty acids labeled in the carboxyl or one of the other odd-numbered carbon positions form glucose or glycogen with isotope almost exclusively in the 3 and 4 carbons, while fatty acids labeled in one of the even-numbered carbon atoms introduce isotope primarily and equally into the 1, 2, 5, and 6 carbons (3, 4).

In order to gather evidence bearing on the utilization of these fundamental intermediary pathways in the human being and to provide data for comparison with pathological states, such as diabetes (1, 5), studies of this type have been commenced by the analysis of distribution of radioisotope in blood glucose after the administration of trace amounts of 1-C^{14}-acetate to four cancer patients with presumably normal carbohydrate metabolism.

MATERIALS AND METHODS

Experimental subjects. General data are given in Table I. All the patients had suffered some weight loss with their disease, but were not wasted and were consuming a regular diet prior to the period of fast stated in Table I. Repeated urinalyses were negative for sugar content and diabetes was absent from the family history in all cases. Liver disease did not exist clinically at the time of study in any case. However, in the case of G. O., one month after study the alkaline phosphatase was found to be elevated to 24 Bodansky units and the serum bilirubin questionably elevated. Liver nodules were palpable before death two months after study. In the case of B. G., autopsy three and one-half months after C^{14} study showed approximately 10 metastatic nodules varying from 0.2 to 0.5 cm. in diameter in the liver. Liver function tests (cephalin flocculation, thymol turbidity, serum bilirubin) were all negative in the case of F. K.

Tracer compound. 1-C^{14}-acetic acid was prepared from $BaC^{14}O_3$ by the Grignard reaction and purified by chromatography on a column of Celite® for use with Subjects B. G., G. O., and J. H. Commercially produced 1-C^{14}-sodium acetate was used for Subject F. K. Degradation of the latter product demonstrated all of the radioactivity to be in the carboxyl carbon.

Subjects B. G., G. O., J. H., and F. K. received 210, 190, 190, and 500 microcuries, respectively, in a single antecubital intravenous injection. The dose of 1-C^{14}-acetate was in each case carried in 0.75 mM of total sodium acetate in 10 ml. of 0.9 per cent NaCl.

Collection and purification of blood glucose. Between two and two and a quarter hours after injection of labeled acetate, there was withdrawn from the opposite antecubital vein approximately 150 ml. of blood from Subjects B. G. and F. K. and *ca.* 500 ml. from G. O. and J. H. Clotting was prevented by using heparinized apparatus. The blood was promptly hemolyzed with five volumes of water and treated with two volumes each of 0.3 N $Ba(OH)_2$ and 5 per cent $ZnSO_4$. A clear solution was separated by centrifugation and filtration and then passed successively through columns of cation (Duolite® C-3) and anion (Duolite® A-4) exchange resins. The deionized eluate was evaporated to dryness *in vacuo* and the residue used in the various degradations of glucose. Quantitative glucose measurements were done either by reduction of copper (6) or by formation of hydroxymethylfurfural (7).

Degradation of glucose. Carbon number one (C-1) of glucose was isolated as formic acid after the action of HBr (8). Conversion of glucose to potassium gluconate followed by reaction of the latter with sodium periodate yielded C-1 as CO_2, C-6 as formaldehyde, and C-2, C-3, C-4, and C-5 together as formic acid (9). Phenylglucosazones were prepared (10) and either combusted totally

[1] This research was supported by the Atomic Energy Commission.

[2] Preliminary findings were presented at the Annual Meeting of the Federated Societies for Experimental Biology, 1956 (1).

TABLE I

Clinical data on experimental subjects

Subject	Age	Sex	Ht. (*cm.*)	Wt. (*Kg.*)	Diagnosis	Period of fast prior to expt.
B. G.	40	M	180	93	Bronchogenic carcinoma	20 hrs.
G. O.	55	M	180	77	Bronchogenic carcinoma	15 hrs.
J. H.	60	M	178	66	Bronchogenic carcinoma	24 hrs.
F. K.	63	F	ca. 156	58	Carcinoma of the colon	15 hrs.

to CO_2 (11) or converted by action of periodic acid to formic acid (C-4 plus C-5), formaldehyde (C-6) and the bisphenylhydrazone of mesoxaldehyde (C-1, C-2, and C-3) (12).

Glucose was fermented by *Lactobacillus casei* to lactic acid, which was degraded by $KMnO_4$ to CO_2 (C-3 plus C-4) and acetaldehyde. The latter was degraded by NaOH and I_2 to formic acid (C-2 plus C-5) and iodoform (C-1 plus C-6) (13, 14). Formic acid was oxidized to CO_2 by mercuric acetate, and iodoform was converted to CO by $AgNO_3$, then to CO_2 by H_2SO_4 and I_2O_5 (13–15). Glucose was fermented by *Leuconostoc mesenteroides* to lactic acid (C-4, C-5, and C-6), ethanol (C-3 and C-2) and CO_2 (C-1) (14, 16). Ethanol was oxidized by H_2SO_4 and $K_2Cr_2O_7$ to acetic acid, from which barium acetate was prepared and pyrolyzed to acetone (9, 14). Treatment of the latter with NaOH and I_2 yielded C-3 (of glucose) as CO_2 and C-2 as iodoform.

Counting techniques. Some organic compounds (phenylglucosazones, bisphenylhydrazones of mesoxaldehyde, and dimedon complexes of formaldehyde) were directly mounted on filter paper and placed beneath the end-window of a Geiger-Mueller tube for measurement of radioactivity. Most of the samples of $C^{14}O_2$ were counted in the same way after mounting of $BaCO_3$ (17). In a later stage of this work (Subject F. K.) $C^{14}O_2$ was measured in the gaseous state by internal proportional counting (18).

RESULTS

Multiple kinds of degradation of glucose were used with Subjects B. G., G. O., and J. H. because the fermentation with *Leuconostoc mesenteroides* (which gives each carbon fraction of glucose separately) was not available until work with F. K. The treatment of glucose with HBr and of phenylglucosazone and potassium gluconate with periodic acid permitted some separation of the carbon fractions which are not obtained in the fermentative method using *Lactobacillus casei.* These procedures also served as a check on the degradation with *L. casei.*

Employment of fermentative methods of degradation introduces the possibility of spurious findings due to activities of bacterial metabolism which may to a minor extent dilute or randomize carbon fractions of glucose. Randomization was noted with *L. casei* action on glucose-1-C^{14} (19) and with *L. mesenteroides* action on glucose containing C^{14} in either the 1, 2, or 6 positions (9, 20). With the latter organism Katz, Abraham, Hill and Chaikoff (20) found 2 to 5 per cent of the total C^{14} in carbon positions other than those expected, while Bernstein, Lentz, Malm, Schambye and Wood (9) found 1 per cent or less.

However, bacterial randomization from Positions 1, 2, and 6 of glucose does not necessarily imply randomization from Positions 3 and 4. Convincing evidence of the validity of small percentages of isotope in 1, 2, 5, and 6, relative to those found in 3 and 4 by the fermentation with *L. mesenteroides,* was presented by Bernstein and co-workers (9), who also measured the C^{14} content of outer glucose carbon fractions by independent chemical methods. Excellent agreement was found between the fermentation values and those found on degradation of potassium gluconate and oxidation of glucobenzimidazole.

The findings of degradation of glucose for all four subjects of the present study are shown in Table II. Virtually all of the total radioactivity of glucose was found in C-3 and C-4 for Subjects B. G. and F. K., and is most likely true although not rigidly proved in the cases of G. O. and J. H. No more than 3.5 per cent of the total glucose C^{14} was found in any of the other carbon positions. There was apparently no significant difference between the activities of C-3 and C-4 for Subjects G. O. and J. H., whereas C-4 had distinctly higher activity than C-3 for Subject F. K.

In all cases whether by fermentative or chemical methods trace amounts, of the order of 1 to 3 per cent of the total glucose C^{14}, were found in the outer carbons of glucose. For Subjects B. G., G. O., and J. H., the absolute amounts of C^{14} measured in these carbons were so low that counting error was high (Table II). From them can be deduced, however, a general impression of

higher activities in C-1 and C-2 than C-5 and C-6. A more conclusive analysis of the pattern of activity in C-1 to C-6 was possible in the case of F. K. due to the combination of a larger amount of C^{14}, a more efficient counting method, and a method of degradation which yields each carbon position separately. Among the four outer carbons, C-2 was found to have the highest activity with C-1, C-5, and C-6 containing lesser amounts of C^{14} in that order.

The overall portion of C^{14} of the carboxyl-labeled acetate which was present in glucose at two hours after injection was calculated on the assumption that there is a body glucose "pool" which has the same concentration as the blood glucose with which it is mixing, and which on the average occupies a hypothetical "space" equivalent to 20 per cent of body weight (21). The values were found to be 1.3 per cent, 1.8 per cent, 1.2 per cent, and 1.7 per cent of the administered dose for Subjects B. G., G. O., J. H., and F. K., respectively. The above estimate is a minimal one for total C^{14} converted to glucose, since it does not include that glucose which was formed and then metabolized within two hours or that which was converted to relatively immobile glycogen.

DISCUSSION

The finding that after injection of 1-C^{14}-acetate virtually all radioisotopic carbon of blood glucose was present in C-3 and C-4 is consistent with previous animal studies and suggests that in the "normal" human liver fatty acid carbons are metabolized via the classical routes, *i.e.*, the Krebs tricarboxylic acid (TCA) cycle and the glycolytic pathway, in the process of conversion to carbohydrate. Figure 1 indicates how the isotope would become thus distributed. It can also be seen from Figure 1, however, that if acetic acid were metabo-

TABLE II

C^{14} distribution in blood glucose following I. V. administration of 1-C^{14}-sodium acetate (200 to 500 microcuries in 1.0 mM) to non-diabetic human subjects

Subject	B.G.		G.O.								J.H.						F.K.	
Method of* glucose degradation	(a)		(a)		(b)		(c)		(d)		(b)		(c)		(e)		(e)	
Carbon Fractions	s.a.†	%‡	s.a.	%	s.a.	%	s.a.	%	s.a.	%	s.a.	%	s.a.	%	s.a.	%	s.a.	%
3,4	300	94	310	96.5														
2,5	11	3.5	6.5	2														
1,6	7	2.5	5.0	1.5														
1							17	2.5	16	2.5			5.5	1	4.5	1	68	1.3
2															6.	1.5	79	1.5
3															180	47.5	1960	38
4															184	48	3030	58
5															3	<1	60	1.1
6					3.5	<1	3.5	<1			2.5	<1	2.5	<1	3	<1	42	.8
1-6					110	100					77	100						
1-3					99	45					74	48.5						
4,5					162	49					104	50						
2-5							164	97					106	98				

* (a) fermentation with Lactobacillus casei, etc.
(b) formation and oxidation of phenylglucosazone, etc.
(c) formation and oxidation of potassium gluconate, etc.
(d) oxidation with hydrogen bromide, etc.
(e) fermentation with Leuconostoc mesenteroides, etc.

† specific activity = counts/minute/milligram carbon: for s.a. values above 50, S.D. of sampling and counting = $< \pm 5\%$; for s.a. values from 20 to 50, S.D. = ± 5 to 10%; for s.a. values below 20, S.D. = ± 5 to 35%; where S.D. (stand. dev.) = $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

‡ per cent of total glucose C^{14} contained in given carbon fractions.

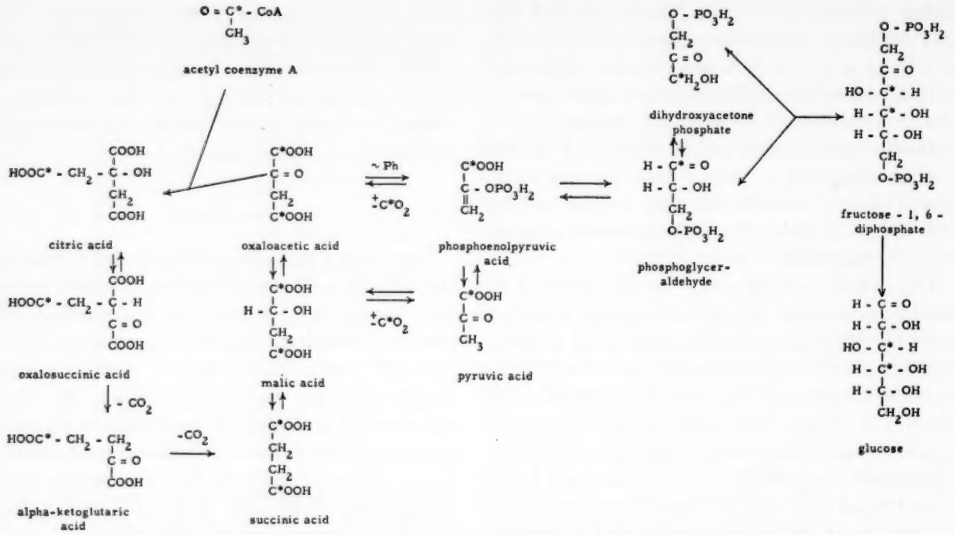

FIG. 1. ABBREVIATED TRICARBOXYLIC ACID CYCLE AND GLYCOLYTIC REACTIONS
Asterisks indicate location of C^{14}.

lized to CO_2 by some other pathway and then fixed into dicarboxylic acids, the distribution of C^{14} in glucose would be the same. It is fundamental to recognize that the net carbon balance in the process of C^{14} introduction from acetate into glucose is zero whether by way of the TCA cycle or by CO_2 fixation, and that there can therefore by these pathways be no true gluconeogenesis from the two-carbon fragments of fat catabolism.

Of interest is the observation that for two subjects (J. H. and G. O.) the C^{14} activity in C-3 was not essentially different than that in C-4, whereas for another subject (F. K.) activity in C-4 was approximately 50 per cent higher than in C-3. Similar variations in symmetry of glucose labeling have been observed by other workers using 2-C^{14}-pyruvate (22) and $C^{14}O_2$ (23) in rats, where the asymmetry appeared to depend upon recent provision of food or unlabeled substrate. Findings from these studies, together with the complementary finding of more C^{14} in C-3 than in C-4 of glycogen glucose after administration of 1-C^{14}-glycerol (24), have led to the hypothesis that unequal labeling between C-3 and C-4 occurs as a result of incomplete equilibration between dihydroxyacetone phosphate and phosphoglyceraldehyde in the presence of appreciable amounts of one or the other unlabeled trioses. Another interpretation has been offered with the demonstration of an exchange of phosphoglyceraldehyde with fructose-di-phosphate by means of a complex between dihydroxyacetone phosphate and aldolase (25). It might also be considered that by operation of various transketolations and transaldolations (26) the first 3 carbons of glucose may undergo rearrangements not shared by the 4, 5, and 6 carbons, and that lack of symmetry could result therefrom.

Further interest attaches to the presence of trace amounts of C^{14} in the 1, 2, 5, and 6 carbons because it is indicative of metabolic reactions not encompassed in the TCA cycle and the glycolytic scheme. Early observations suggested trace activity in the outer carbons of glycogen glucose after administration of carboxyl-labeled fatty acid (3) or of radioactive bicarbonate (27). More recent and definitive studies (9, 23, 28) have indicated the presence of a small amount of activity (5 per cent or less of the glucose C^{14}) in each of the outer carbons of glucose from rat liver glycogen after formation from $NaHC^{14}O_3$. There has generally been observed more in C-1 and C-2 than C-5 and C-6.

The oxidative route for glucose metabolism

with reformation of hexose via the pentose phosphate cycle has been discussed by Wood (29) and Racker (26) among others as a means of extension of tracer, primarily to C-2, from originally 3,4-labeled glucose. Further extension to Positions 1,5, and 6 may take place by recycling in the glucose oxidative pathway and by conversion of hexose to triose with subsequent CO_2 fixation and randomization in the Krebs cycle. Additionally, extension from C-3 to both of the first 2 carbons of glucose may take place by reactions of transketolation and transaldolation operating reversibly and separately from the complete oxidative cycle for glucose (thus accounting for activity in C-1 similar to that in C-2 and higher than in C-5 and C-6).

Other mechanisms, such as CO_2 fixation with pentose phosphate (labeling C-1 of hexose), are possible but less likely (23, 30), as would presumably be the recently-elucidated glucuronic acid-xylulose pathway (31), which would transfer carbon from C-4 to C-5 of hexose. Still other reactions, which would label the outer carbons along with net formation of glucose from 1-C^{14}-acetate, are mentioned in a subsequent paper (5). The findings from the present study of traces of C^{14} in the outer carbon of glucose would seem primarily to suggest the operation in the human liver of the oxidative and the nonoxidative reactions of the pentose phosphate cycle. The low level of activity of these reactions relative to the rate of glucose synthesis via the Embden-Meyerhof pathway appears to be about the same as that found for normal rat (9, 23, 27) and mouse (29) liver. It may be noted that in glycogen from rabbit liver slices incubated with $NaHC^{14}O_3$, Topper and Hastings (12) found no C^{14} in positions other than 3 and 4, nor did Abraham, Chaikoff and Hassid (32) after injecting 1-C^{14}-tripalmitin into diabetic dogs and analyzing urinary glucose.

The overall fraction (average of 1.5 per cent) of the administered C^{14} found in the theoretical "pool" of miscible body glucose at two hours is of the same general order of magnitude as that calculated by Strisower, Chaikoff and Weinman (10) for extracellular glucose of the rat at one hour after injection of 6-C^{14}-tripalmitin (0.6 per cent for normal and 2.3 per cent for diabetic) as well as that found by Abraham and co-workers (32) to be excreted in the urine by the diabetic dog within 24 hours after injection of 1-C^{14}-tripalmitin (4.7 per cent). Unfortunately, there are no available data for the human on the amount to be expected simply from $C^{14}O_2$ fixation, so it cannot in this way be determined whether an additional amount of C^{14} was incorporated from acetate via particular pathways such as the tricarboxylic acid cycle.

At the time this investigation was initiated it was deemed necessary to use cancer patients as subjects representative of the "normal" and nondiabetic human in order to be able to use sufficient amounts of C^{14}. It has recently been emphasized (21, 33) that some patients with cancer tend to have an alteration of carbohydrate metabolism in the direction of the diabetic type. Whether this situation has had any influence on the present findings cannot be stated. However, the subjects were manifestly not in the acute diabetic state of patients with whom they have been compared (5). In another way the presence of a neoplasm (ascites cell tumor of mice) has been shown to change the pattern of labeling of liver glycogen, but not appreciably unless the tumor cells have first access to the labeled precursor as a result of its intraperitoneal injection (34). It can indeed be questioned generally whether the pattern of label in blood glucose from the intact organism reflects only hepatic metabolism, since some other organ or tissue may have taken part in distribution of the isotope either before or after the formation of glucose in the liver or by extra-hepatic (*e.g.*, renal) gluconeogenesis. It may be noted in this connection that intermediates of the pentose phosphate cycle have been found to occur in human hemolysates (35).

SUMMARY

The nature of various metabolic pathways used in the transfer of carbon from fatty acid to carbohydrate in the human being has been investigated by means of C^{14} tracing. The experimental subjects were four patients with carcinoma (of the lung or colon) who had no grossly evident abnormality in carbohydrate metabolism. They were fasted for 15 to 24 hours prior to the test. Single intravenous injections of trace amounts of carboxyl-labeled sodium acetate (200 to 500 microcuries) were followed two hours later by collec-

tion of blood from which glucose was isolated and subsequently degraded to individual or groups of carbon fractions by various bacteriological and chemical methods.

As in previous similar studies with animals, virtually all of the radioisotopic carbon of glucose was present in the 3 and 4 positions of the molecule. Whereas in two patients the activity of these two positions was essentially equal, in one patient there was more activity in C-4 than C-3. In each of the other positions (1, 2, 5, and 6) there was a slight amount of radioactivity, of the order of 1 to 2 per cent of the total glucose C^{14} in each position. There appeared to be generally more in C-1 and C-2 than C-5 and C-6. An average of 1.5 per cent of the administered C^{14} was estimated to be present in free body glucose at the time of sampling.

The finding of primarily 3,4-labeled glucose is consistent with the operation of the tricarboxylic acid cycle and the glycolytic pathway as the principal routes for passage of carbon from fatty acid to carbohydrate. The presence and relative amounts of tracer in the 1, 2, 5, and 6 carbons suggest the operation of reactions of the pentose phosphate cycle.

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PATHWAYS OF CARBOHYDRATE FORMATION IN MAN. II. THE EFFECT OF DIABETES AND GLUCOCORTICOID ADMINISTRATION ON ISOTOPE DISTRIBUTION IN GLUCOSE FROM SUBJECTS GIVEN 1-C^{14}-ACETATE [1, 2]

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The pattern of glucose labeling after administration of 1-C^{14}-acetate in nondiabetic human subjects has been established (1, 2) and found to be essentially the same as observed with animals using various odd-carbon-labeled fatty acids as precursors (3, 4). Such isotopic studies have supported the concept of transfer of fatty acid carbon to glucose by way of the tricarboxylic acid cycle and the glycolytic reactions, *i.e.*, the classical route of glucose synthesis from various noncarbohydrate precursors. Whether these same reactions are solely utilized in those diabetic states in which overproduction of glucose is a possible contributing factor remains a question conceivably answerable by similar study with position-labeled compounds. Paramount to the question is the possible utilization of a pathway which, unlike the classical route of glucose synthesis, would afford a net gain in carbohydrate from fat. Major changes in the kind or quantity of available substrates, available coupling reactions, and so forth, might conceivably alter existing pathways or set in operation others which would eventuate in gluconeogenesis from fat in significant amounts.

The present study of isotope distribution in glucose after administration of trace amounts of 1-C^{14}-acetate has been conducted with various types of diabetic patients, including some with fully developed ketosis and acidosis and others in whom has been tested the effect of administration of an adrenal glucocorticoid. In confirmation of another such study in the diabetic dog (5), the findings in brief have not disclosed the abnormal utilization of pathways other than those previously assumed to account for glucose formation in the diabetic as well as the normal state. The total amount of C^{14} converted to glucose, however, has again suggested overproduction of glucose, particularly in acute ketotic diabetes.

[1] This research was supported by the Atomic Energy Commission.

[2] The findings were presented in part at the Annual Meeting of the Federated Societies for Experimental Biology, 1956 (1).

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EXPERIMENTAL SUBJECTS

Subject V. P. This patient was an 11 year old white female; weight, 37 Kg.; height, 139 cm. Her diabetic condition had been known to be present since the age of six. Severe acidosis was present at the time of discovery of diabetes, and insulin shock occurred in the course of initial regulation. The patient had required at various times since onset from 4 units to 50 or 60 units insulin per day. During the present hospital admission it was difficult to control glycosuria, hyperglycemia and occasional acetonuria without development of mild signs and symptoms of hypoglycemia. Four days prior to the isotopic study, the daily insulin course was changed from 30 units regular insulin plus 35 units protamine zinc insulin to regular insulin four times daily (25-8-8-8 units). On the day prior to study the usual 1,800 calorie diet (149 Gm. carbohydrate, 93 Gm. protein, 92 Gm. fat) was substituted by one containing 100 Gm. fat and 100 Gm. protein (hamburger and butter pats) evenly divided among three meals. No food was given after 6 p.m. On this day the patient received 20 units regular insulin at 8 a.m. and 5 units at 12 a.m. and no further insulin prior to study. At 5 p.m. the blood sugar was 192 mg. per cent, plasma CO_2 was 24.4 mEq. per L., and the urine strongly positive for acetone. At the time of injection of isotope the next day the patient was clinically acidotic, the blood sugar was 362 mg. per cent, and the plasma CO_2 was 6.7 mEq. per L. (Figure 1).

Subject H. R. This patient was a 38 year old white female; weight, 57 Kg.; height, 160 cm., with an onset of diabetes 11 years before. Regulation of diabetes by diet and insulin (30 to 50 units daily) was relatively easy until the advent two years before the present study of a pregnancy, during and after which bouts of acidosis and insulin shock were characteristic. For 10 days prior to study the patient received no long-acting insulin, but regular insulin four times daily (approximately 12-12-12-4 units). On the day before the planned study, the patient's customary 2,100 calorie diet (217 Gm. carbohydrate, 95 Gm. protein, 92 Gm. fat) was changed to

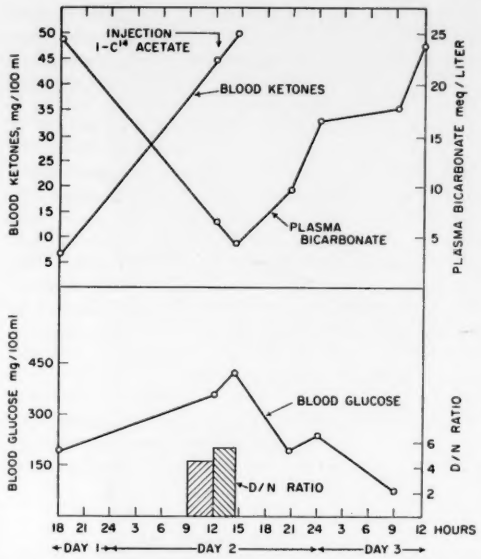

FIG. 1. LAB DATA FOR PERIOD OF C^{14} STUDY WITH LABILE DIABETIC SUBJECT V. P., FEMALE, AGED 11

100 Gm. fat and 100 Gm. protein as with Subject V. P. No insulin was given after the eight a.m. dose of 12 units. The urine was 4 plus positive for sugar and acetone during the day and the patient became clinically acidotic late in the day. Due to the clinical state and blood chemistry (blood sugar equaled 664 mg. per cent, plasma CO_2 equaled 13.6 mEq. per L., Figure 2) it was considered advisable to proceed with injection of the isotopic compound at one a.m.

Subject M. M. This patient was a 34 year old white female; weight, 71 Kg.; height, *ca.* 160 cm. Detection of diabetes three and one-half years prior to study was accompanied by findings of obesity ("buffalo type"), hirsutism, sparse scalp hair, and hypertension (230/160). Diabetes was established by the glucose tolerance test. An insulin tolerance test indicated moderate insulin resistance. Diabetes was readily controlled by diet alone and occasionally in conjunction with insulin. The blood hemoglobin was 15.5 Gm. per cent. There were no eosinophils found either on differential or quantitative eosinophil count. The 17-ketosteroids in a 24 hour urine sample were 3.06 mg. per cent and the plasma 17-OH steroids were 25 μg. per cent basal going to 87 μg. per cent upon stimulation with adrenocorticotrophic hormone (25 units intravenously in six hours). Cardiac decompensation and hepatomegaly characterized the later course of this patient, as did some renal impairment (4 plus albuminuria, blood urea nitrogen of 38 mg. per cent). Prior to isotopic study the patient was receiving a balanced 1,000 calorie diet and no insulin. At the time of C^{14} injection the patient had been fasting approximately 18 hours since the previous evening. Blood sugar at the time of injection was 218 mg. per cent.

Subject D. M. This patient was a 59 year old white male; weight, 64 Kg.; height, 168 cm., whose diabetes was detected 12 years before. The disease was controlled by a 30 pound weight reduction, and no insulin was required until five months previous to admission, when intermittent glycosuria forced the use of 25 to 40 units NPH insulin daily. In the hospital, glycosuria and hyperglycemia were quite readily controlled with a 2,000 calorie diet (240 Gm. carbohydrate, 80 Gm. protein, 80 Gm. fat) and 25 to 40 units insulin. Arteriosclerosis and neuropathy moderately affected the lower extremities; there was 1 plus albuminuria and punctate retinal aneurysms were present. The patient received no insulin after the usual dose of NPH on the morning 72 hours prior to isotopic study. After the evening meal 40 hours prior to study food was withheld. Four plus acetonuria appeared at nine p.m. of the day before study and remained continuously until after the experimental period. Ketonuria cleared after feeding was resumed and before insulin was given. There was no glycosuria before or during the isotope experiment. The blood sugar was 181 mg. per cent at time of study and the plasma CO_2 was 23 mEq. per L. (Figure 3). Nine hours and again at three hours prior to study the patient received 30 mg. prednisone (delta-1-cortisone) orally. On another occasion this dose of steroid produced in D. M. a drop of plasma eosinophils from 112 to 1.5 per cu. mm.

Subject P. R. This patient was a 55 year old white male; weight, 82 Kg.; height, 169 cm. Diabetes was

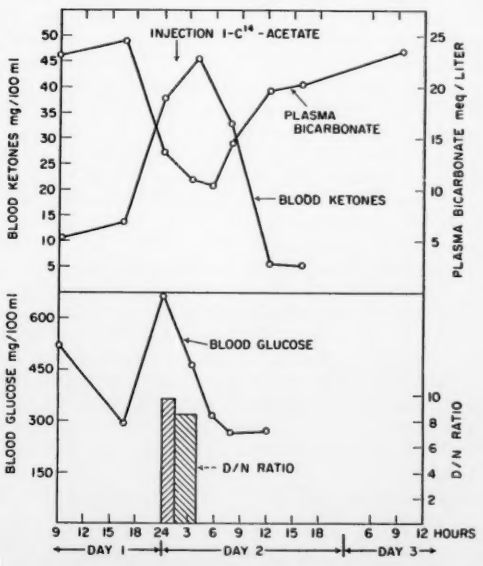

FIG. 2. LAB DATA FOR PERIOD OF C^{14} STUDY WITH LABILE DIABETIC SUBJECT H. R., FEMALE, AGED 38

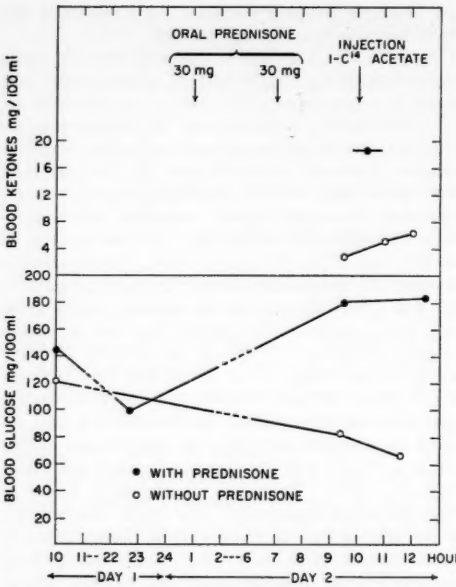

FIG. 3. LAB DATA FOR PERIOD OF C^{14} STUDY WITH STABILE DIABETIC SUBJECT D. M., MALE, AGED 59

discovered five years previously during the course of work-up for hypertension. He had no symptoms of diabetes at that time and was treated with diet alone. His fasting blood sugar gradually rose to about 250 mg. per cent, and one month prior to isotopic study he was started on 20 units Lente insulin. During two weeks of hospital admission prior to study, his diabetes was readily controlled with a daily dose of 35 units Lente insulin, while the patient was on a diet of 2,200 calories (226 Gm. carbohydrate, 100 Gm. protein, 101 Gm. fat) with low-sodium content. During this time his weight decreased by approximately 4 Kg. The patient had no signs or symptoms of degenerative changes accompanying diabetes. The blood pressure was 218/126, and the liver was palpable one fingerbreadth below the costal margin. Seventy-two hours before study the patient received the last dose of Lente insulin and 48 hours before study, a final dose of regular insulin. Food was withheld for 40 hours prior to isotope injection. At nine and at three hours prior to study he received 30 mg. prednisone by mouth. There was no glycosuria before or during the study, but the urine became strongly positive for acetone at four p.m. of the day preceding study and remained so until the latter part of the next day after restoration of food and insulin. At the time of isotope injection the blood sugar was 172 mg. per cent and the CO_2 was 26 mEq. per L. (Figure 4).

METHODS

In each case 80 to 100 microcuries of 1-C^{14}-sodium acetate (with 1.0 mM reagent sodium acetate for carrier in 15 to 25 ml. of 0.9 per cent NaCl) was given by rapid antecubital intravenous injection. Blood samples were taken at intervals for analysis of glucose by the methods of Miller and Van Slyke (6) or Mendel, Kemp, and Myers (7), analysis of ketone bodies by the method of Michaels, Margen, Liebert, and Kinsell (8), and CO_2 of plasma by the method of Peters and Van Slyke (9). Nonprotein nitrogen in the urine was measured according to a slight modification of Peters and Van Slyke (10) and Kingsley (11). Approximately two hours after injection of the labeled acetate a blood sample of about 100 ml. was withdrawn from the opposite arm. From this sample glucose was isolated as previously described (2), except that for two subjects (P. R. and D. M.) the glucose was additionally purified by chromatography with acetone-water on cellulose columns (12) following elution from ion exchange columns. The glucose was degraded by fermentation with *Leuconostoc mesenteroides* and subsequent chemical steps followed by analysis of C^{14} in gaseous carbon dioxide as elsewhere described (2). Phenylglucosazones were prepared and analyzed as before (2).

RESULTS

The blood levels of ketone bodies and plasma CO_2 in Subjects V. P. (Figure 1) and H. R. (Figure 2) leave no doubt that in these subjects the iso-

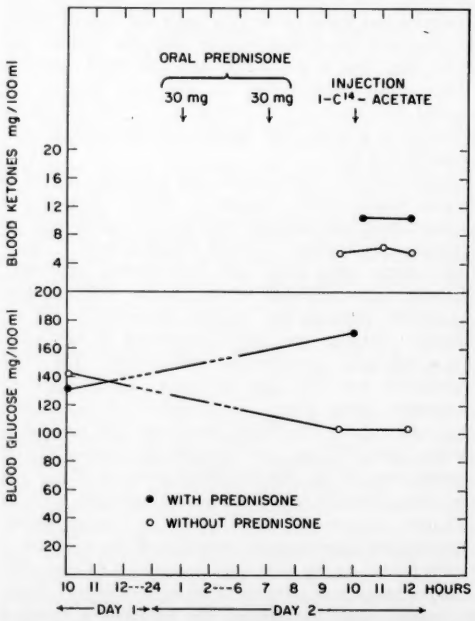

FIG. 4. LAB DATA FOR PERIOD OF C^{14} STUDY WITH STABLE DIABETIC SUBJECT P. R., MALE, AGED 55

topic acetate was being metabolized at a time of acute ketotic diabetes. It was considered necessary to produce this condition since certain hypotheses of abnormal routes of formation of glucose involve acetoacetic acid as an intermediate compound (*vide infra*), and since a similar ketosis was present in another human study which claimed to demonstrate gluconeogenesis from fat on the basis of high urinary D/N ratios (13). The purpose of modifying the diet in these two patients to one of fat and protein on the day before study was to eliminate dietary carbohydrate as a source of urinary glucose and thus make possible the interpretation of a high urinary D/N ratio. If there were no source of circulating carbohydrate other than protein and fat, then gluconeogenesis from fat would be suggested by a urinary D/N ratio above a certain value, theoretically as high as about 6 (13–15), but actually perhaps no more than 3 or 4 (16), assuming the utilization of some glucose by the tissues (15, 17). Both Subjects V. P. (Figure 1) and H. R. (Figure 2) exceeded the lower range, but only H. R. exceeded the higher estimate. It is doubtful, however, if the period of adjustment to a change in diet was sufficiently long to deplete liver glycogen, particularly in the case of H. R., to allow for any valid interpretation in regard to gluconeogenesis from fat.

It was considered that the other three diabetic patients, M. M., D. M., and P. R., might serve to test the condition of abnormal gluconeogenesis as related to glucocorticoid action. The diabetes of M. M. was associated according to clinical signs with adrenal corticoid hyperactivity, though laboratory findings did not definitely confirm (or deny) this. With Subjects D. M. and P. R., who were typically stable, obese diabetics, an elevation of blood sugar occurred following the administration of the synthetic glucocorticoid, delta-1-cortisone. Under the same conditions without glucocorticoid administration the blood glucose levels did not rise (Figures 3 and 4). In both instances, *i.e.*, with and without delta-1-cortisone, both patients displayed a similar degree and time of onset of ketonuria (*cf.* Experimental Subjects) and mild ketonemia (Figures 3 and 4). The subjection of Patients D. M. and P. R. to a 40 hour fast prior to study was again for the purpose of interpreting a D/N ratio unaffected if possible by consumed or stored carbohydrate, but the moderate degree of hyperglycemia was not accompanied by any urinary output of glucose.

In Table I are given the results of degradation of glucose and isotopic analysis of each carbon fraction for all of the diabetic subjects. Just as was found with nondiabetic human subjects (1, 2), virtually all (96 to 98 per cent) of the glucose C^{14} was located in the carbon 3 and carbon 4 positions. Activity in carbon 4 was in each case higher than that in carbon 3 but to a variable extent (10 to 50 per cent). Specific activities of carbons 1, 2, 5, and 6 varied from 0.2 to 1.6 per cent of the total glucose C^{14}. The validity of these low percentage activities has been previously ascertained (2, 18). The low absolute levels of radioactivity of these carbon fractions and resultant high counting errors (Table I) would yet seem to permit the conclusions that activities of carbons 1, 2, 5, and 6 relative to carbons 3 and 4 are approximately the same as in nondiabetic subjects and that, as in the latter case (2), carbons 1 and 2 have activities quite similar to each other and generally higher than the activities of carbons 5 and 6. Blood glucose, collected from Subjects V. P. and H. R. two hours after C^{14} administration, was also degraded with less complete results, but showed essentially the same distribution of C^{14} as was found with urinary glucose.

From the C^{14} activities of phenylglucosazones of blood glucose and the blood glucose level at two hours after isotope injection, the total amount of C^{14} in free body glucose [presumed to be equally distributed in a hypothetical "space" equal to 20 per cent of total body weight (2)] was calculated. From these values it was estimated that the fraction of injected C^{14} present in total free body glucose at two hours after administration of 1-C^{14}-acetate was as follows: V. P. equals 4.2 per cent,[4] H. R. equals 3.6 per cent,[4] M. M. equals 1.7 per cent, D. M. equals 1.8 per cent, and P. R. equals 2.4 per cent. Values for V. P. and H. R. (the two unstable and strongly ketotic diabetics) are considerably higher than those found in four nondiabetic subjects (average of 1.5 per cent, range of 1.2 to 1.8 per cent) (2). Values for the other three (stable) diabetic subjects are not signifi-

[4] For V. P. an additional 0.7 per cent of the injected C^{14} was present in urinary glucose excreted during the first two hours after administration, and for H. R., an additional 0.2 per cent.

Table I

Distribution of C^{14} in glucose* following I.V. administration of 1-C^{14}-sodium acetate (50 to 100 microcuries in 1.0 mM) to diabetic human subjects

Glucose carbon fraction	Subject V.P.**		Subject H.R.**		Subject M.M.†		Subject D.M.†		Subject P.R.†	
	s.a.††	%†††	s.a.	%	s.a.	%	s.a.	%	s.a.	%
C-1	10	.6	5	.4	4	.8	9	.8	10	1.4
C-2	12	.7	8	.7	9	1.6	7	.6	10	1.4
C-3	841	47.	496	43.	258	47.	491	42.	272	39.
C-4	917	51.	626	55.	273	50.	648	56.	410	58.
C-5	5	.3	5	.4	5	.9	4	.4	3	.4
C-6	5	.3	3	.3	1	.2	2	.2	2	.2

*For V.P., urinary glucose excreted 2.5 to 7 hours, and for H.R., urinary glucose excreted 3 to 4 hours after C^{14} administration; for M.M., D.M., and P.R. blood glucose collected 2 hours after administration (variably diluted with carrier glucose prior to isotopic analysis).

**Unstable diabetics in ketoacidosis.

†Stable diabetics.

††Specific activity = disintegrations/minute/milligram carbon; standard deviation of sampling and counting, where S.D. = $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$, was 5 percent or less for C-3 and C-4, 5 to 25 percent for C-1 and C-2, and 15 to 100 percent for C-5 and C-6.

†††Per cent of total glucose C^{14} contained in each carbon fraction.

cantly higher on the average than for nondiabetic subjects. Furthermore, in the "control" studies of D. M. and P. R. without steroid administration, the amounts of C^{14} found in blood glucose two hours after C^{14}-acetate administration were essentially the same as with steroid administration, *i.e.*, 1.9 per cent and 2.4 per cent for D. M. and P. R., respectively. Thus, in spite of the apparent hyperglycemic effect of the synthetic glucocorticoid no more acetate radiocarbon was converted to glucose. A factor of uncertainty in the calculation is a question as to whether the ketotic, acidotic diabetics (V. P. and H. R.) could be considered to have a contracted extracellular fluid space (and therefore contracted volume of distribution of glucose) as a result of partial dehydration. Such an effect would render the estimation of amount of C^{14} in glucose unduly high.

DISCUSSION

The controversial evidence on gluconeogenesis from fat in mammalian species has been repeatedly reviewed and discussed (4, 14, 19–22). To summarize in brief, while classical balance studies have generally failed to demonstrate extra formation of carbohydrate from fat fed to experimentally diabetic animals (19, 21), other quantitative studies with perfused organs and tissue slices (14, 23–25) as well as intact animals (14, 26, 27) and humans (13) have indicated from consideration of respiratory quotients, dextrose/nitrogen ratios, and various other data on carbon balance, that under certain experimental conditions some fatty acid as well as protein (amino acids), glycerol, or lactic acid may contribute to an increase in amount of carbohydrate.

Isotopic findings, such as the formation of primarily 3, 4-labeled glucose from carboxyl-labeled fatty acids (2–4), have generally supported the original concept that the main intermediary metabolic pathways permit the conversion of fatty acid carbon to glucose but without net gain in amount of carbohydrate. Nevertheless, there have been reasons for postulating that under some special and abnormal conditions a different pattern of labeling in glucose might be produced which would disclose a pathway for gluconeogenesis from fatty acids. For instance, 2-C^{14}-labeled acetone was

found to label the 1, 2, 5, and 6 carbons of glucose (28) and the 2 carbon of alanine (29) to such an extent as to suggest a major disposal of acetone by conversion possibly through propanediol (30) to a 3 carbon compound such as pyruvate. Since acetoacetic acid may be actively decarboxylated to acetone (31), a series of reactions, such as in Figure 5, could be conceived for net formation of glucose accompanied by an abnormal pattern of C^{14} distribution in the glucose derived from a carboxyl-labeled fatty acid. It seemed worthwhile to put this hypothesis (and others mentioned below) to a clinical test with diabetic patients in a state of advanced ketosis, in which high tissue concentrations of ketone bodies in the liver (and perhaps kidney) might result in marked acceleration of a pathway like that shown in Figure 5.

Alpha oxidation of beta-hydroxybutyric acid was suggested twenty years ago (23) in order to explain increase in amount of carbohydrate and lactic acid after presentation of butyric acid, beta-hydroxybutyric acid, and alpha, beta-dihydroxybutyric acid to surviving tissues (muscle, kidney, spleen, brain, and liver) of cats and dogs. Such a pathway would presumably label the outer carbons of glucose after administration of a carboxyl-labeled fatty acid. Omega (terminal methyl carbon) oxidation of butyric acid to succinic acid was early suggested (24) to be responsible for an observed net formation of carbohydrate and would also form 1, 2, 5, and 6-carbon-labeled glucose, from 1-C^{14}-acetate, as would a similar conversion of acetoacetic or beta-hydroxybutyric acids. In ketosis one would expect the latter to be more likely than the former according to current concepts of a block in diabetes between "ketone bodies" and butyrate (32). Another reaction which might eventually form in net quantity 1, 2, 5, and 6-labeled glucose from 1-C^{14}-acetate is the reversal of the oxidative decarboxylation of pyruvate. Though this occurrence is most unlikely from thermodynamic considerations, some evidence was obtained for potential reversibility of the immediate reaction of decarboxylation in a pig heart muscle system (22).

The finding of the normal labeling pattern in glucose from blood and urine of diabetic patients given 1-C^{14}-acetate indicates that none of the above postulated pathways was responsible for glucose formation in significant amount in the liver. The suggested pathways involving "ketone bodies" were not undetected because of failure of transfer of C^{14} to acetoacetate and beta-hydroxybutyrate, since the labeled (1 and presumably 3) carbons of the latter in the urine were found to have C^{14} activities of the same order as the 3 and 4 carbons of glucose from the same patients (unpublished observations). The results therefore coincide with similar findings from the use of 3-labeled butyrate in normal rat liver (3) and cat heart muscle (33), 1,3-labeled acetoacetate in normal rat liver (28) and 3-labeled acetoacetate in dog heart muscle (34). Moreover, the results are parallel to those obtained in the only other similar study in a diabetic organism, *i.e.*, the totally pancreatectomized dog (5), in which tracer was detected only in the 3 and 4 carbons of glucose after administration of 1-C^{14}-tripalmitin. In the latter instance, the animals were heavily glycosuric, but the state of ketosis was not defined as in the present investigation with diabetic patients.

It should be recognized that the conventional

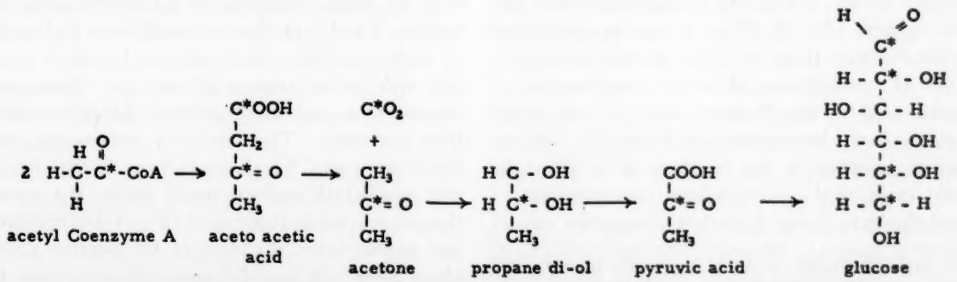

FIG. 5. REACTIONS FORMING GLUCOSE FROM ACETATE VIA ACETOACETATE AND ACETONE
Asterisks indicate location of C^{14}.

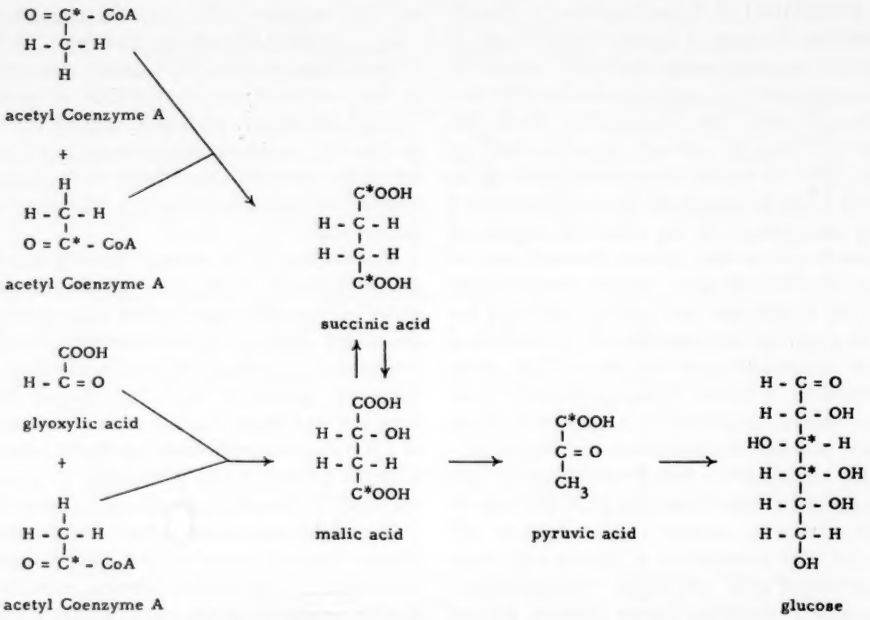

FIG. 6. REACTIONS FORMING GLUCOSE FROM ACETATE VIA "TAIL-TO-TAIL" CONDENSATION TO SUCCINATE AND CONDENSATION WITH GLYOXALATE TO MALATE

Asterisks indicate location of C^{14}.

labeling pattern found with odd-carbon-labeled fatty acid does not exclude all possible means of net formation of glucose. The early suggestion (35) of a "tail-to-tail" condensation of two molecules of acetate to form succinate (upper section of Figure 6) and then obviously net carbohydrate is a reaction consonant with present isotopic data. Evidence has been produced for an enzyme from rat (36) and rabbit (37) tissues which cleaves succinate in this manner, but reversibility in mammalian species, if it occurs, is apparently slow and inconsistent (36, 38, 39). A reaction postulated quite recently from evidence in microorganisms (40, 41) and plants (41) is a condensation of acetic acid [5] with glyoxylic acid to form malic acid, as in the lower section of Figure 6. This reaction, whether or not involved in a "glyoxylic acid cycle" (41), would form net quantities of carbohydrate from 2 carbon fragments of fat metabolism and is consistent with, though not specifically indicated by, present data on glucose labeling. A recent attempt to demonstrate the requisite enzymatic reactions in animal tissues was unavailing, however, even under the condition of excessive fat feeding (42).

The trace amounts of C^{14} found in the 1, 2, 5, and 6 carbons of glucose from patients in this study are not considered to have been derived by any of the mechanisms suggested above, because if so by initial formation of 2,3-labeled pyruvate carbons 5 and 6 of glucose would have had equal or higher activities than carbons 1 and 2, [(2) and *vide infra*], unless glycerol or dihydroxyacetone is a product of acetone oxidation rather than pyruvate. The similarity in amount and distribution of C^{14} in these carbons to that found with nondiabetic patients would suggest the operation, as previously mentioned (2), of the oxidative and nonoxidative reactions of the pentose phosphate cycle. If any difference from findings in control subjects exists at all, it is in the direction of generally lower activities relative to carbons 3

[5] Active derivative (with CoA) would be more precise, as for many intermediates mentioned in this discussion, but such have been omitted for purposes of brevity and clarity.

and 4, which could be merely a reflection of increased glucose synthesis relative to an unchanged rate of reactions responsible for C^{14} in the 1, 2, 5, and 6 carbons. In view of various observations of the effect of diabetes (43, 44), cortisone (44), and starvation (43–45) on activity of the pentose phosphate cycle, it is perhaps noteworthy that no major differences in the amount and pattern of 1, 2, 5, and 6-glucose carbon labeling were detected as a result of nutritional and hormonal changes in the present study.

Reasons for difference in C^{14} content of carbons 3 and 4 with regard to mechanisms of glucose formation have been discussed in a preceding publication (2). Current theory, on the basis of nonequilibration of triose units, would predict disparity of carbons 3 and 4 in proportion to the amount of substrate available for glycolytic intermediates. It is not seemingly possible to interpret the variable differences of carbon 3 and carbon 4 activity found in the diabetic or nondiabetic patients on any nutritional or hormonal basis. The larger differences between carbons 3 and 4 in the 40 hour fasted subjects than in those fasted for considerably shorter periods does not coincide with the current theory on the basis of fasting *per se*, since the fasting state would presumably tend to equilibration of dihydroxyacetone phosphate and phosphoglyceraldehyde.

Despite uncertainty in the estimation of amounts of C^{14} in the free body glucose of various patients as discussed above (*cf.*, Results), it would nevertheless seem that in the labile, strongly ketotic diabetics two to three times as much (*ca.* 4 per cent versus 1.5 per cent) of the administered C^{14} appeared in glucose as in the nondiabetic subjects (2), while little if any more than the amount in controls appeared in the glucose of stable diabetic subjects with glucocorticoid excess. The results with labile diabetics, at least, are quite in agreement with the findings from normal and alloxan-diabetic rats given 2-C^{14}-acetate and 6-C^{14}-tripalmitin (46).

Due to the involvement of many unknown factors, such as the relative amounts of glycogen formed in the various patients, it would be inadvisable to draw conclusions from the C^{14} data regarding rates of total carbohydrate synthesis. Certainly the differences in general C^{14} content of glucose do not provide information as to the utilization of expected or unusual pathways. If, indeed, an excess of glucose was formed in the labile diabetics, the findings do not seem to bear out the usual concept that overproduction of glucose is more characteristic of diabetes of adrenal origin than of diabetes of the pancreatic, insulin-deficient type. The possibility should not be overlooked that in the acidotic diabetics natural adrenal factors were operating more effectively than the administered synthetic steroid (47). Evidence on this point includes observations from isotopic data that a sevenfold greater than normal production of glucose resulted from administration of cortisone to rats, whereas only a twofold increase occurred in alloxan-diabetic rats (48). Findings from the present study may be viewed as more compatible with glucose turnover as observed in normal and diabetic humans (17) in whom overproduction of glucose was suggestively correlated with degree of ketosis in unstable diabetics. In view of the still possible utilization of unusual pathways discussed above and perhaps others not presently recognized, the question as to whether fatty acids may contribute in some unique way to excessive gluconeogenesis in certain diabetic situations may not yet be considered fully resolved.

SUMMARY

Production of glucose in various states of clinical diabetes has been investigated with five patients. Two of these were young, labile diabetics who were in a condition of marked ketosis and acidosis, one was a stable diabetic with some clinical evidence of increased adrenal activity, and two were middle-aged, obese, stable diabetics to whom a large dose of prednisone (delta-1-cortisone) had been administered shortly before study. Mild ketosis was present in the latter two subjects.

Each patient was injected intravenously with a trace amount of 1-C^{14}-acetate followed in two hours by collection of blood and in some cases various urinary samples for glucose analysis. Degradation of glucose (by fermentation with *Leuconostoc mesenteroides*) and analysis of C^{14} content of each carbon position showed virtually all of the glucose C^{14} (96 to 98 per cent) to be in the 3 and 4 carbons, as has been previously observed with animals and nondiabetic humans. These findings are consistent with the operation

of the tricarboxylic acid cycle and/or fixation of carbon dioxide followed by reversal of glycolytic reactions as the means of appearance of C^{14} in glucose. The occurrence of the conventional glucose labeling pattern fails to provide evidence for utilization of special metabolic pathways by which gluconeogenesis (net gain in carbohydrate) from fat could occur in possibly predisposing nutritional and hormonal conditions. If certain pathways, principally involving ketone bodies as intermediates, had been operational, then the 1, 2, 5, and 6 carbons of glucose would have been substantially labeled. The findings do not exclude all possible reactions which could result in gluconeogenesis from fat.

Small amounts of tracer (less than 2 per cent of the total glucose C^{14}) were present in each of the 1, 2, 5, and 6 carbons in such a proportion as mainly to suggest derivation by reactions of the pentose phosphate cycle. C^{14} was present in these carbon positions in approximately the same pattern and amount as with nondiabetic humans.

In the labile diabetics with marked ketosis approximately 4 per cent of the administered C^{14} was estimated to be present in free body glucose two hours after injection, while in the stable diabetics with or without glucocorticoid administration about 2 per cent was found in glucose at this time. Since nondiabetic humans in corresponding studies showed an average of 1.5 per cent of administered C^{14} in glucose, the data are suggestive, though by no means conclusive, of an increased rate of carbohydrate synthesis from acetate in the labile, acidotic diabetics. The data would not distinguish between the utilization of special or common pathways of glucose synthesis.

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THE EFFECTS OF ACID–BASE BALANCE ON THE DIURESIS PRODUCED BY ORGANIC AND INORGANIC MERCURIALS [1]

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Changes in acid-base metabolism have been recognized for many years as one of the most important factors influencing the magnitude of the diuretic response to organic mercurials. Diuresis is potentiated during metabolic acidosis and is suppressed during metabolic alkalosis. The mechanism by which these effects are mediated has been the subject of a number of studies. In general, two separate points of view have been considered: first, that they are attributable to changes in pH which presumably either modify the metabolic fate of the mercurial [4] or alter the affinity of a renal component for the drug; and secondly, that the responsiveness of the kidney varies with alterations in the ionic composition of the plasma which are independent of pH. Evidence in support of the latter thesis has been presented by Axelrod and Pitts (1) in studies on respiratory and metabolic acidosis. These authors have postulated that the concentration of chloride in the glomerular filtrate is the major factor that determines the effect of organic mercurials. Although this thesis has been widely accepted in recent years, there are a number of observations which cannot be explained by it. These include the diuretic response observed during ammonium nitrate acidosis (2), chronic ammonia chloride administration (3), and the extracellular alkalosis of potassium depletion (4).

Experiments were therefore designed to reexamine the hypothesis that pH itself might influence the metabolic fate of organic mercurials. Benesch and Benesch have described a two step reaction between mersalyl and dimercaprol (5). The first reaction results in the formation of a dimercaptide and is not pH dependent; the second reaction results in the rupture of a carbon to mercury bond and is pH dependent. Using this *in vitro* reaction as a model, it was decided to compare the diuretic response to an organic mercurial with that to inorganic mercury under different conditions of acid-base balance. Thus, it was postulated that inorganic mercury, not requiring the rupture of the carbon to mercury bond, might be effective during acute metabolic alkalosis, whereas an organic mercurial would be relatively ineffective.

METHODS

Two types of experiments were carried out on trained mongrel female dogs. In one type standard clearance techniques were employed with continuous intravenous infusion, while in the other metabolism cages were used for collecting urine for three hours after drug administration. Since the clearance studies lasted for a similar three hours, the results obtained from these two types of studies have been combined where applicable. Each dog served as her own control, with an interval of at least one week between experiments. Dogs were maintained on a standard diet consisting of Purina Chow Dog Checkers.

In the clearance experiments, animals were fasted 12 to 18 hours before each experiment, but allowed free access to water. Alkalosis was produced by the rapid infusion of 300 to 500 ml. of isotonic sodium bicarbonate at 14 ml. per minute until the urine became alkaline and the plasma bicarbonate was elevated to the range of 26

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[4] The older literature speaks of the "dissociation of mercurials" under the influence of acids. The process really involves the rupture of a covalent carbon-mercury bond which is not an ionic dissociation in the usual sense.

TABLE I

Comparison of meralluride and mercuric-cysteine in metabolic alkalosis, metabolic acidosis and under normal conditions *

Condition	Drug†	C_{Cr}	Plasma Na	Plasma K	Plasma HCO_3	Plasma Cl	$\Delta U_{Cl}V$ ‡	Peak $\Delta U_{Cl}V$‡§
		ml./min.		*mEq./L.*			*mEq./3 hr.*	*μEq./min.*
Dog: Biggy, 30 Kg.								
Alkalosis	Meralluride	101			30.2	104	− 3¶	+ 29
Alkalosis	Mercuric chloride	105			31.6	99 (−5.0)‖	+ 70¶	+ 965
Normal	Meralluride	109			22.2	109	+ 58**	+ 923
Normal	Mercuric chloride	166			21.4	110	+ 97	+1,104
Dog: Teddy, 19 Kg.								
Alkalosis	Meralluride	70	150	2.7	26.0	104 (−5.2)	− 0.4	+ 182
Alkalosis	Mercuric-cysteine	53	155	3.4	26.8	103 (−6.0)	+ 85	+ 747
Normal	Meralluride	62	156	3.4	21.4	111	+ 71	+ 863
Normal	Mercuric-cysteine	61	145	4.0	21.7	113	+151	+1,570
Acidosis	Meralluride	74			15.2	117	+127	+1,468
Acidosis	Mercuric-cysteine	55			17.7	116	+172	+1,522
Dog: Mischief, 20 Kg.								
Alkalosis	Meralluride	52	154	3.4	29.2	105 (−5.0)	+ 16	+ 146
Alkalosis	Mercuric-cysteine	75	157	3.4	26.8	112 (−6.0)	+ 51	+ 634
Normal	Meralluride	62	155	3.2	21.9	109	+ 24¶	+ 485
Normal	Mercuric-cysteine	77	147	3.2	22.8	108	+109	+1,222
Acidosis	Meralluride	88	151	3.4	18.4	113	+ 88	+1,059
Acidosis	Mercuric-cysteine	77	146	3.6	17.3	119	+112	+1,078
Dog: Satin, 20–23 Kg.								
Alkalosis	Meralluride	72	149	3.0	27.3	104 (−5.0)	+ 13	+ 153
Alkalosis	Mercuric-cysteine	64	154	3.4	28.0	106 (−7.0)	+ 56	+ 681
Normal	Meralluride	69	152	3.7	19.6	114	+ 83	+ 772
Normal	Mercuric-cysteine	64	148	4.0	19.0	115	+181	+1,674
Acidosis	Meralluride	95	145	4.7	14.9	121	+255	+2,425
Acidosis	Mercuric-cysteine	83	146	3.8	15.6	119	+255	+2,182

* Values for plasma electrolytes and for creatinine clearance (C_{Cr}) are averages of periods before and for 40 minutes after administration of mercurial.

† Drugs were administered in a dose of 1 mg. of Hg per Kg. for mercuric salts, and 2 mg. of Hg per Kg. for meralluride.

‡ $\Delta U_{Cl}V$ represents the chloride excretion in excess of control excretion for the duration of the experiment.

§ The values in this column indicate the peak rate of chloride excretion above control levels.

‖ Figures in parentheses opposite values for plasma chloride refer to change in chloride concentration (mEq. per L.), resulting from bicarbonate infusion.

¶ 140 minutes.

** 100 minutes.

to 29 mEq. per L. In experiments during normal conditions of acid-base balance 300 ml. of isotonic sodium chloride was infused, and serum chloride and bicarbonate remained within the normal range. Acidosis was produced by the administration of 10 grams of ammonium chloride by stomach tube in two divided doses on the afternoon prior to the experiment. During the clearance experiment itself, the acidotic dog received the same priming and sustaining infusion as in the experiments carried out under normal conditions. In all experiments following completion of the initial infusion, the rate was reduced to 2 ml. per minute using a solution identical in electrolyte concentration to that initially employed. Creatinine was added to all infusions to maintain constant plasma levels of about 20 mg. per cent. Thirty minutes were allowed for equilibration and two 20 minute control periods were then taken. The diuretic was injected intravenously over a five minute period. Meralluride,[5] 2 mg. of mercury per Kg., was used as the standard organic mercurial. Inorganic mercury was administered as 1 mg. of mercury per Kg. in the form of mercuric chloride in the early experiments. Later this was given with a tenfold excess of cysteine, mixed just prior to injection. This mixture is referred to as mercuric cysteine. All dogs receiving this agent were treated with dimercaprol for the subsequent two to three days.

In the "cage" experiments, fasted dogs were catheterized in the morning, returned to their cages, and were permitted no further food or water. At the end of two hours, a control collection of urine was obtained by catheterization, following which the mercurial was administered slowly intravenously. The urine was collected

[5] Generously supplied by Drs. H. L. Daiell and H. L. Friedman, Lakeside Laboratories, Inc., Milwaukee 1, Wisc.

for the next three hours, at the end of which the bladder was emptied by catheterization. In control experiments, without diuretics, electrolyte excretions were essentially constant during this five hour period. In both the "clearance" and "cage" types of experiments, results have been expressed in most instances as the change in urinary chloride excretion, obtained by subtracting the control rate of excretion from that after the diuretic.

The creatinine clearance (C_{Cr}) was used as the measure of the glomerular filtration rate; creatinine was determined by the alkaline picrate method using trichloroacetic acid filtrates of plasma. Sodium and potassium were determined on a Patwin flame photometer with an internal lithium standard; plasma carbon dioxide was determined on samples collected anaerobically by the manometric method of Van Slyke; urine carbon dioxide, by the volumetric method of Van Slyke. In all experiments, results have been expressed as the concentration of bicarbonate (HCO^-_3) in the plasma, obtained, by subtracting carbonic acid from total CO_2. In some experiments, when pH was determined, carbonic acid was calculated from the Henderson-Hasselbalch equation; in others, pCO_2 was assumed constant at 40 mm. Hg. Urine and blood samples were collected anaerobically under oil and pH was determined immediately with a Beckman Model G pH meter. Chloride was originally measured by the method of Wilson and Ball (6) and in later experiments by the electrometric titration method of Cotlove (7).

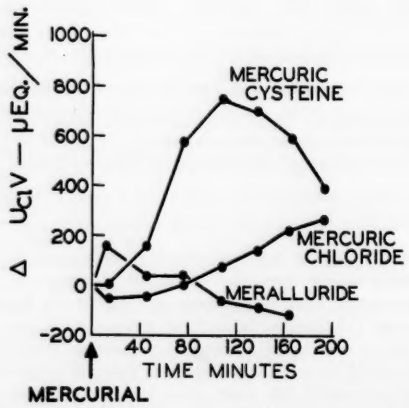

FIG. 1. CHLORIDE EXCRETION IN RESPONSE TO THREE DIFFERENT FORMS OF MERCURY IN AN ALKALOTIC DOG

The data are from experiments on the same dog (weight, 19 Kg.). The amount of bicarbonate infused and the plasma electrolyte concentrations were essentially the same in each experiment. The ordinate is a measure of the change in the rate of chloride excretion relative to premercury periods. The dose of meralluride was 2 mg. of Hg per Kg.; of $HgCl_2$ and Hg cysteine, 1 mg. of Hg per Kg.

RESULTS

In preliminary experiments on acute metabolic alkalosis comparison was made between the effects of meralluride (2 mg. of Hg per Kg.) and inorganic mercury as mercuric chloride (1 mg. of Hg per Kg.). In confirmation of many previous observations the organic mercurial was ineffective. In contrast, mercuric chloride had a marked chloruretic action in some dogs, while in others this was less marked and appeared late in the experiment (see Table I and Figure 1). It was considered that this inconsistent response might be related either to cardiac toxicity or to binding of mercuric ions to plasma proteins and to early distribution to organs other than the kidney. Since Farah and Maresh (8) have shown that the administration of a monothiol, such as cysteine, reduces cardiac toxicity without alteration in diuretic potency, inorganic mercury was administered as mercuric cysteine in subsequent experiments. As shown in Table I and Figure 1, mercuric cysteine uniformly produced a chloruresis during alkalosis.

Since the acute metabolic acidosis produced by ammonium chloride potentiates the chloruretic action of organic mercurials, experiments were undertaken to determine whether the action of inorganic mercury (as mercury cysteine) would be similarly increased. Table I summarizes the results in three dogs. Under normal conditions meralluride produced an average increase in chloride excretion of 2.9 mEq. per Kg. over a three hour period. With ammonium chloride acidosis, this value increased to 7.4 mEq. per Kg. With mercuric cysteine, there was little potentiation with acidosis, the average values being 7.1 mEq. per Kg. for normal and 8.6 mEq. per Kg. for acidosis.

In the above experiments, mercuric cysteine and meralluride were administered in the arbitrary doses of 1 and 2 mg. of Hg per Kg., respectively. While the greater potency of mercuric cysteine during alkalosis was consistent with the initial hypothesis, it was not anticipated that mercuric cysteine would be a more potent diuretic than the organic mercurial under conditions of normal acid-base balance. Previous observations have indicated that organic mercurials are much more effective diuretics than inorganic mercury. Indeed, the relative potency of the organic and inorganic

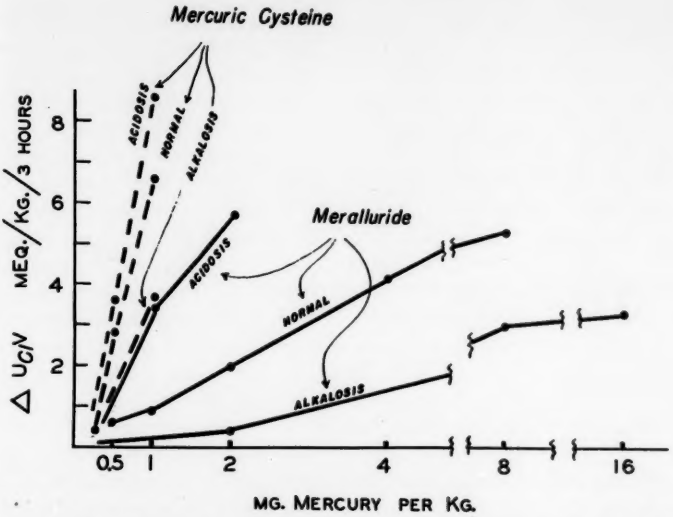

FIG. 2. THE EFFECT OF ACID-BASE BALANCE ON CHLORURETIC RESPONSE TO MERALLURIDE AND MERCURIC CYSTEINE

Each point represents the mean of three or more experiments. Plasma bicarbonate levels for alkalotic dogs fell between 26 to 30 mEq. per L.; for normal dogs, between 19 to 25 mEq. per L.; and for acidotic dogs, between 15 to 18 mEq. per L. This figure includes all applicable data from "cage" and "clearance" experiments.

forms of mercury has been considered as evidence that the organic compounds act as intact molecules rather than by the rupture of a carbon to mercury bond (9). Experiments were therefore done to compare mercuric cysteine and meralluride over a wide dosage range. As shown in Figure 2, the maximal increase in chloride excretion was about 8 mEq. per Kg. over the three hour period of observation. This corresponds to the loss of approximately one-third of the chloride in extracellular fluid, and is also equivalent to the excretion of approximately 20 per cent of the filtered chloride at the peak of the diuretic response.

Several points of interest are to be emphasized from the data depicted in Figure 2: *1*) Mercuric cysteine, at 1 mg. of Hg per Kg., produced the maximal response. Larger doses were not administered because of the possibility of late toxicity. *2*) During alkalosis, the chloruresis due to mercuric cysteine is about one-half the maximal response. *3*) During acidosis, meralluride was potentiated to such an extent that it became almost as effective as mercuric cysteine. However, mercuric cysteine was not significantly potentiated by acidosis. *4*) Under normal conditions and during alkalosis, meralluride was much less potent than the inorganic form of mercury. *5*) While meralluride at 2 mg. of Hg per Kg. is virtually inactive during alkalosis, moderate chloruresis is obtained if the dose is greatly increased (8 or 16 mg. of Hg per Kg.). On the basis of reaction kinetics, this finding would be anticipated if diuretic activity depended on a pH dependent reaction involving the organic mercurial. Estimation of the relative potencies of these agents is as follows: During acidosis, 0.5 mg. of Hg of mercuric cysteine is equivalent to about 1 mg. of Hg of meralluride (ratio, 1:2). Under normal conditions, 0.5 mg. of Hg of mercuric cysteine is equivalent to about 3.5 mg. of Hg of meralluride (ratio, 1:7). During alkalosis, 1 mg. of Hg of mercuric cysteine is equivalent to about 16 mg. of Hg of meralluride (ratio, 1:16). It is obvious, therefore, that the organic mercurial is a relatively weak diuretic and that it approaches the potency of inorganic mercury only under acidotic conditions.

Comparison was also made of mercuric cysteine and chlormerodrin (Neohydrin®), which was se-

lected because of reports indicating that it is more potent than meralluride (10). Under normal conditions, an approximately equivalent response was obtained with 0.5 mg. of Hg per Kg. as mercuric cysteine, 1.0 mg. of Hg per Kg. as chlormerodrin, and 3.5 mg. of Hg per Kg. as meralluride. In addition, during alkalosis, chlormerodrin (2 mg. of Hg per Kg.) resembled meralluride in that it failed to produce a chloruresis.

The magnitude of diuresis has been considered in terms of the urinary excretion of chloride since the primary effect of mercurials is generally believed to be chloruretic (1). In all experiments, changes in urinary sodium paralleled those of chloride; variations in potassium excretion were minor and inconsistent. In some experiments, an increase in bicarbonate excretion was noted; this was related to slight fluctuations in the serum bicarbonate concentration.

In the present experiments the peak response was obtained between one and two hours and, by the third hour, the diuresis had subsided. As shown in Table I, the same relationships were observed whether the results were expressed as the change in chloride excretion over this entire period or as the peak rate of excretion. Others have shown that the absolute magnitude of chloruresis is partially determined by the volume of extracellular fluid (11).

Although it has been shown that an acute reduction in filtration rate to 75 per cent of the control value blocks the response to organic mercurials (12), it is apparent from the results shown in Table I that comparable spontaneous day to day variations in the filtration rate have no observable effect on diuresis. Although multiple doses of mercurials were administered, no significant changes in filtration rate were observed over an extended period of time, and, in individual experiments, there were no consistent changes immediately after the administration of either drug.

Since the acute production of either metabolic acidosis or metabolic alkalosis involves changes in plasma chloride concentration as well as in pH, an experiment was undertaken in an attempt to modify acid-base balance while keeping plasma chloride level essentially constant. This was accomplished by adding to an infusion of sodium chloride a fixed amount of other sodium salts, either bicarbonate, nitrate or sulfate. The results obtained in two dogs are shown in Table II and Figure 3. In all experiments either a normal or

TABLE II

*Effect of meralluride in dog loaded with sodium chloride in combination with another sodium salt**

	Dog B, 27 Kg.				Dog C, 26 Kg.		
Infusions†	NaCl $NaNO_3$	NaCl $NaNO_3$	NaCl Na_2SO_4	NaCl $NaHCO_3$	NaCl Na_2SO_4	NaCl $NaHCO_3$	NaCl $NaHCO_3$
Meralluride *2 mg. Hg/Kg.*	+	0	+	+	+	+	0
C_{Cr}, ml./min.	142	182	152	154	111	110	94
Plasma Cl^-, mEq./L.	111	102	113	114	112	112	111
Plasma HCO_3^-, mEq./L.	19.8	23.0	21.6	26.1	21.3	28.1	26.8
Venous pH	7.37		7.46	7.49	7.40	7.48	7.48
Urine pH	4.9		6.0	7.7	6.3	7.7	7.7
Water balance, ml.	+934	+615	+963	+980	+1,290	+931	+896
Chloride balance, mEq.	+113	+96	+149	+137	+196	+126	+133
Max. change, $\Delta U_{Cl}V$, μEq./min.	+1,662	+380	+1,040	+350	+1,320	+493	+570
Total change, $\Delta U_{Cl}V$, mEq./2 hr.	+120	+23	+90	+34	+105	+38	+46

* Values given for creatinine clearance (C_{Cr}), plasma electrolytes, and plasma and urine pH are the average for two 20 minute periods before and two 20 minute periods after mercurial administration. Water and chloride balance represent the difference between total input and output at the time of drug administration. In all experiments, the control rate of chloride excretion was calculated for the 40 minutes before mercurial or a corresponding period when no drug was given. In the control experiments (without mercurials) the change in $U_{Cl}V$ was calculated for an interval of time corresponding exactly to the postmercurial period. In all experiments the plasma chloride level at the time of mercurial administration was 0 to 3 mEq. per L. higher than the preinfusion value.

† Dogs were loaded with 1 L. of a primary intravenous infusion containing 150 mEq. of NaCl, 25 mEq. of $NaHCO_3$, and 125 mEq. of the test salt; infusion time, 50 to 60 minutes. The sustaining infusion at about 10 ml. per minute contained 115 mEq. per L. of NaCl and 60 mEq. per L. of test salt. Meralluride was administered 70 minutes after the start of the sustaining infusion.

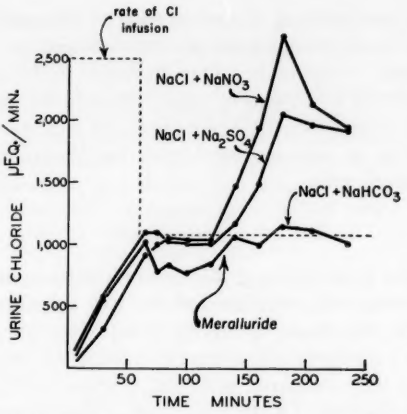

FIG. 3. THE EFFECT OF THE INFUSION OF CHLORIDE PLUS ANOTHER ANION ON THE CHLORURETIC RESPONSE TO MERALLURIDE

The dotted line represents the average rate of primary and sustaining chloride infusions which varied not more than 10 per cent from one experiment to the other. The protocol for these experiments and the clearance and plasma electrolyte data are summarized in Table II (Dog B).

an increased chloride load was delivered to the tubules. Under these conditions the alkalosis produced in the sodium bicarbonate experiments was associated with a slight elevation of the serum chloride. This alkalosis was accompanied by a marked decrease in the chloruretic response to meralluride; a normal chloruresis was obtained when the additional anion load was either nitrate or sulfate.

DISCUSSION

The present evidence relating to the effect of acid-base balance on mercurial action may be summarized as follows. Consistent with the "chloride load hypothesis" are the following: *1*) In the comparison of respiratory and metabolic acidosis, potentiation of mercurial diuresis is correlated with hyperchloremia rather than with the pH of the plasma (1).[6] *2*) Although mercuric cysteine is not significantly potentiated by ammonium chloride acidosis, its action is somewhat decreased by a moderate metabolic alkalosis with plasma bicarbonate levels in the range of 26 to 29 mEq. per L. *3*) An acute depression of the glomerular filtration rate is associated with a corresponding decrease in the amount of chloride filtered without any accompanying change in the pH of body fluids. While mercurials become inactive under these circumstances, the physiological disturbances are of such a nature that this type of experiment may well not be relevant to the present problem.

The following observations *cannot* be readily interpreted in terms of the "chloride load hypothesis": *1*) Metabolic alkalosis and acidosis have a profound effect on the potency of organic mercurials and considerably less of an effect on the potency of inorganic mercury given as the mercuric cysteine complex. *2*) The simultaneous administration of acetazolamide inhibits organic mercurials (13) but our preliminary experiments indicate only a negligible effect on mercuric cysteine. *3*) When various sodium salts are administered along with sodium chloride, the chloruretic response to organic mercurials is inhibited during metabolic alkalosis despite slight elevations in the plasma chloride level. *4*) In the dog, a decrease in plasma chloride of 5 to 7 mEq. per L. is uniformly associated with refractoriness to organic mercurials when this is produced by isotonic sodium bicarbonate infusions, but a normal mercurial diuresis is obtained if a similar degree of hypochloremia is produced by simple hemodilution, *i.e.*, by the administration of water (14).[7] *5*) During potassium depletion there is a normal response to mercurials despite hypochloremia (4). *6*) Ammonium nitrate potentiates mercurial diuresis without an increase in plasma chloride (2). *7*) Potentiation of mercurials by ammonium chloride occurs in chronic experiments in the absence of hyperchloremia (3).

If the diuretic action of mercurials involves a pH dependent reaction, does this occur in the cells of the kidney or in the urine within the tubule? Our knowledge of renal intracellular pH is so

[6] There is no *a priori* reason why plasma pH must be implicated in the action of mercurials. The pH at the intra-renal site of action of the drug is probably the critical parameter, and there is no evidence to suggest the changes there will be the same as in plasma. The acidity of urine is a parameter that correlates well with mercurial effectiveness (see below).

[7] A similar observation has been reported by Farah and Koda (15) who obtained a chloruretic response to mercurials following reduction of plasma chloride to values as low as 80 mEq. per L. by the administration of hypotonic solutions.

fragmentary and so indirect that this question cannot be answered at present. Since the electrolyte metabolism of the kidney is so different from that of other tissues, it would appear unreasonable to attempt to define renal mechanisms by extrapolation from other tissues. It is generally believed that the site of mercurial action is on the tubular cell (or on its membrane), and there is good evidence that mercurials are taken up by the cells of the kidney and thence secreted into the tubular urine (16–18). Hence it might be logical to conceive of a pH dependent reaction as occurring within the cell itself. However, there is no *a priori* reason why the rupture of a carbon to mercury bond could not occur within the tubular urine. This might then be followed by a subsequent reaction between mercuric ions and the immediately adjacent cellular membrane. In this connection the findings reported by Nicholson (19) are of interest. He employed a histochemical modification of the earlier experiment of Macallum and Campbell (20) and interpreted his findings as indicating a zone of marked acidity in the region of the brush border of the proximal tubule.

Mercuric cysteine was initially employed in these experiments because of the possibility that the inconsistent results obtained with mercuric chloride might be attributable to cardiac toxicity. In retrospect no evidence of cardiac toxicity was observed with mercuric chloride when given slowly intravenously in the small doses that were employed. A more plausible interpretation of the relative potencies of mercuric chloride and mercuric cysteine involves their distribution within the body. Tissue analyses have not been performed. However, it was observed that the initial rate of excretion of mercury in the urine was about twice as great with mercuric cysteine as with mercuric chloride (21); presumably this may be a function of the amount taken up by the kidney from the plasma.

The relative ineffectiveness of mercuric chloride, when compared to organic mercurials, has previously been considered as evidence against the thesis that the diuretic activity of the organic compounds is attributable to the mercuric ion itself (9). However, the argument loses validity by the demonstration that the mercuric cysteine complex is more potent than the organic mercurials thus far tested. Further evaluation of this question involves a consideration of the metabolism and structure-activity relationships of the organic compounds. Our findings, to be presented in a subsequent paper, support the thesis that diuretic activity results from the rupture of a carbon to mercury bond with the liberation of mercuric ions.

SUMMARY

The potentiation of mercurial diuretics by acid-forming salts was studied by two approaches. First, the effects of varying doses of meralluride and a mercuric salt, mercuric cysteine, on chloruresis were compared in normal, acidotic (NH_4Cl) and alkalotic ($NaHCO_3$) dogs. The mercuric salt is a much more potent chloruretic and it is influenced to a much smaller extent by changes in acid-base balance than is meralluride. This finding is interpreted as indicating that a major part of potentiation by acid-forming salts is related to the relative ease of release of mercuric ions from organic mercurials in acid media.

Second, dogs received hypertonic infusions which kept plasma chloride concentration and glomerular filtration rate elevated, while the acid-base balance was varied by the infusion of other anions, *i.e.*, nitrate, sulfate and bicarbonate. Under these circumstances, with almost identical chloride load, bicarbonate inhibited the mercurial effect, while nitrate and sulfate did not. This finding emphasizes the role of pH in determining mercurial effectiveness.

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EFFECTS OF METHYL TESTOSTERONE ON THYROID FUNCTION, THYROXINE METABOLISM, AND THYROXINE-BINDING PROTEIN

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Studies on the effect of androgenic steroids on thyroid function are difficult to evaluate because of a number of apparent discrepancies. Money, Kirschner, Kraintz, Merrill, and Rawson (1), using castrate rats on a low iodine diet, found that testosterone propionate had no influence on thyroid weight or on thyroid weight: body weight ratio, but did increase the radioiodine uptake. Kochakian and Evans (2) recently reported that this form of testosterone failed to influence the uptake or release of radioiodine by the thyroid of the castrate rat. Burris, Bogart, and Krueger (3), using heifers and young steers, found that testosterone propionate produced an increased gain in weight; furthermore, the thyroids of the treated animals weighed more, contained less colloid, and exhibited an increase in thyroid cell height. Voitkevich (4) is cited as stating that the goiters produced by propylthiouracil in guinea pigs and cocks are very considerably decreased by simultaneously administered methyl testosterone, and that propylthiouracil goiters are larger in castrate than in normal cocks.

In man, Kinsell, Hertz, and Reifenstein (5) demonstrated that tesosterone propionate reversed the negative nitrogen balance and creatinuria of two hyperthyroid patients, although without having clearcut effect on their hypermetabolism. Methyl tesosterone, on the other hand, induced a less sustained positive nitrogen balance, increased the creatinuria, and appeared to exacerbate the hyperthyroidism. Recently, Keitel and Sherer (6) observed that the administration of methyl testosterone to dwarfed children and to adults with metastatic breast carcinoma, produced decreases in the serum protein-bound iodine and in radioiodine uptake. The present study was undertaken to investigate the mechanism of these effects. Studies in the same subjects of thyroid radioiodine clearance, of thyroxine-binding protein (TBP), and of the rate of degradation of thyroxine, might serve to differentiate direct effects on the thyroid from extrathyroidal mechanisms.

METHODS

The subjects were three adult males and one prepubescent girl. Pertinent data are included in Table I. The subjects, with the exception of B. B., were hospitalized during the study and were on reasonably constant activity and diet. After a suitable control period, methyl testosterone was given in a dosage of 100 mg. daily (25 mg. four times daily *per os*) for seven weeks. The times of the studies in relation to duration of treatment are listed in the tables. *In vivo* counting was done with a collimated scintillation counter having a one-eighth inch lead window and placed at 40 cm. from the suprasternal notch. The collimation was such that the circle seen at 40 cm. which included the 50 per cent maximal counting rate was 12 cm. in diameter. *In vitro* counting of plasma and urine samples was done in a well-type scintillation counter.

Radiothyroxine disappearance rates, organic iodine pools, and iodine degradation were measured, using the slope and intercept method of calculation (7). I^{131}-labeled L-thyroxine [2] (5 to 10 micrograms, 50 to 100 microcuries per dose) was given intravenously, and samples of serum were assayed daily for two weeks thereafter. Thyroidal reaccumulation of iodide was blocked with methimazole, 80 mg. daily.

Basal metabolism was measured weekly. Protein-bound iodine (PBI) in serum was measured three times in each period of testing, using the modified method of Barker and Humphrey (8) or a modification of the Zak, Willard, Myers, and Boyle (9) procedure. Thyroxine-binding protein (TBP) of the serum was assayed by the method described earlier (10) and calculation of the free thyroxine level was made using equations evaluated

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[2] Obtained from Abbott Laboratories, assayed for purity by chromatography and repurified, if necessary, to a purity of at least 90 per cent.

TABLE I

Clinical data in subjects

Patient	Age	Sex	Diagnosis	Thyroid status
B. B.	40	M	Diabetes mellitus	Euthyroid
C. H.	12	F	Primary mental deficiency Epilepsy	Euthyroid
L. W.	16	M	Primary mental deficiency	Euthyroid
D. W.	31	M	Primary mental deficiency	Euthyroid

elsewhere (11). Iodide clearances were obtained from intravenous radioiodide tracers by measuring the radioactivity over the neck, in heparinized plasma, and in urine at numerous intervals for six hours (12). To determine the fraction of the neck counts due to extrathyroidal sources, a separate radioiodide tracer was given to each of three of the subjects at the conclusion of the study and after his gland had been "blocked" with 2 grams of sodium iodide. Two grams of sodium iodide is a dose sufficient to dilute the I^{131} so that if as much as 2 or 3 mg. of iodine is accumulated by the thyroid, only approximately 0.1 per cent of the I^{131} will be accumulated. From the curves of neck and plasma radioactivity so obtained, corrections for extrathyroidal activity corresponding to the plasma levels of the initial, unblocked tracer were found. Extrathyroidal neck radioactivity was then calculated on the basis of the level of I^{131} in plasma. Clearances were calculated in the usual manner from two to six hours after the dose of radioiodide.

RESULTS

Clinical changes

None of the patients showed any symptoms or signs of hypo- or hyperthyroidism, and none had any toxic symptoms from the methyl testoterone or Tapazole®. The three patients weighed repeatedly gained 1 to 2 Kg. each during the treatment period, and lost the weight within two weeks after discontinuing the drug. Basal metabolism rates proved to be uninterpretably variable. Serum cholesterol values rose in all patients during treatment and returned toward control levels after the medication was discontinued (Figure 1). In the case of D. W., values had not yet returned to pretreatment levels four weeks after treatment had been stopped.

Iodide metabolism

Thyroidal uptake of radioiodide, thyroidal iodide clearance, and renal iodide clearance were measured before methyl testosterone and after 47 to 51 days on the drug (Table II). All values were within the normal range. Although the data for each of the subjects (except L. W. on testosterone) were internally compatible in that the 24 hour uptake could be reasonably predicted from the corresponding thyroidal and renal clearances, the differences observed between control and treatment values revealed no consistent effect of the drug.

Thyroxine metabolism

The changes in hormonal iodine levels in serum are shown in Table III. There was a small but consistent reduction in protein-bound iodine after 28 to 29 days of treatment with testosterone. Although one value was in the hypothyroid range, in general the changes were not so marked as those found by Keitel and Sherer (6). The mean pretreatment value was 4.9 micrograms per cent; dur-

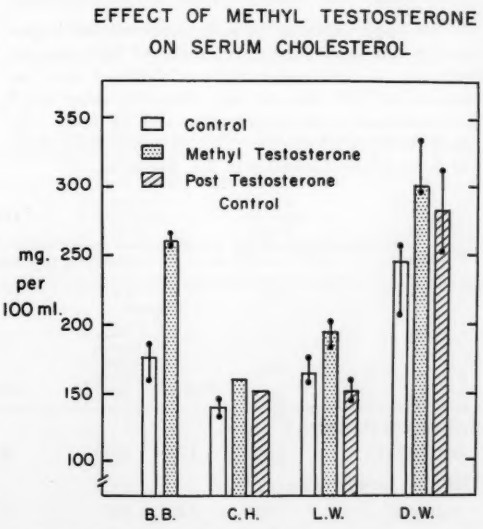

FIG. 1. EFFECT OF METHYL TESTOSTERONE IN THE SERUM CHOLESTEROL

The height of the bars represents the mean level of cholesterol for the period; the vertical lines represent the range of values for the period. No post-treatment values were obtained on Patient B. B.

TABLE II

The influence of methyl testosterone on thyroidal uptake and thyroidal and renal clearance of iodide

	Patient B. B.		Patient C. H.		Patient L. W.		Patient D. W.	
	Control	Methyl testosterone (51)*	Control	Methyl testosterone (47)	Control	Methyl testosterone (48)	Control	Methyl testosterone (48)
Radioiodide uptake, %								
3 hours	29	16	20	10	12	12	15	16
6 hours	36	21	18	12	14	14	17	22
24 hours			24	13	19	18	27	33
Thyroidal iodide clearance, ml./min./1.73 M.²	39	20†	20	20	9	13	8	18
Renal iodide clearance, ml./min./1.73 M.²	43	35	56	100	43	36	27	34

* The numbers in parentheses indicate days on testosterone at time of study.
† Since the neck: plasma radioiodine ratio could not be determined in B. B. after iodide "block" (see text), the average of the values obtained in the other subjects was employed.

ing treatment it was 3.7 micrograms per cent. For consistency in presentation of the data in Table III, protein-bound iodine has been converted to molar concentration of thyroxine.

The thyroxine-binding capacity of the thyroxine-binding protein (TBP) was normal in all patients before treatment (mean equals 2.4×10^{-7} M).[3] During treatment, the concentration of TBP sites fell to abnormally low levels (mean equals 0.98×10^{-7} M). Because of the relatively greater decrease in thyroxine-binding protein than in PBI the calculated level of free thyroxine rose, although the small change in B. B. and C. H. is of questionable significance (*cf.* Discussion). The mean control free thyroxine was 7.0×10^{-11} M; the mean during treatment was 10.9×10^{-11} M. In all but C. H., the value during treatment was above the range seen in a series of normal adults (11, 13). No qualitative alteration in the distribution of thyroxine among the serum proteins, as determined

[3] This value, expressed as molar concentration, is presumably equivalent to the concentration of TBP sites for thyroxine. If the number and availability of sites per molecule of TBP does not vary, then this value would be proportional to the concentration of TBP itself. Although there is no evidence on this point, these terms will be used interchangeably in this discussion.

TABLE III

The influence of methyl testosterone on iodine metabolism: Protein-bound iodine, thyroxine-binding protein, and free thyroxine levels in serum

	Patient B. B.		Patient C. H.		Patient L. W.		Patient D. W.	
	Control	Methyl testosterone (29)*	Control	Methyl testosterone (28)	Control	Methyl testosterone (29)	Control	Methyl testosterone (29)
Endogenous thyroxine,† $\times 10^{-7}$ M‡	1.4	0.90	0.77	0.44	0.89	0.71	0.78	0.71
TBP sites for thyroxine, $\times 10^{-7}$ M	3.0	1.5	1.7	0.66	2.1	0.67	2.6	1.1
Free thyroxine, $\times 10^{-11}$ M	8.9	10	7.3	8.2	7.1	16	4.6	9.9

* In parentheses is the number of days on testosterone at time of study.
† Calculated from the protein-bound iodine on the assumption that it consists entirely of thyroxine.
‡ Conversion factor: Protein-bound iodine (micrograms per cent) equals $5.03 \times 10^7 \times$ molar concentration thyroxine.

by electrophoresis at pH 8.6, was induced by the testosterone.

The fractional rate of thyroxine disappearance was increased in all patients during treatment, from a mean of 13 per cent per day to 19 per cent per day after 23 to 27 days of treatment (Table IV). The extrathyroidal organic iodine pool was unchanged in three patients, reduced in one (B. B.). The higher fractional degradation resulted in an increased absolute degradation of hormonal iodine in all patients, although the differences were small and questionably significant in two cases. The mean pretreatment was 53 micrograms iodine per day per 1.73 M.^{2}; the mean during treatment was 68. Following cessation of testosterone treatment, the rate of thyroxine disappearance fell and the amount of iodine degraded per day decreased despite an apparent rise in the extrathyroidal organic iodine pool. The significance of the pool change is uncertain. Although not given in the table, the volume of distribution of thyroxine increased during treatment in all cases and fell after cessation of testosterone. These changes are consistent with known effects of testosterone on fluid retention.

Data for two patients who were studied at intervals after cessation of methyl tesosterone are presented in Figure 2. The minimal concentrations of TBP sites for thyroxine were found in the blood obtained seven days after treatment was stopped. Subsequently there was a slow return toward normal levels, although the concentration of TBP sites was still subnormal at 35 days. The levels of free thyroxine rose during treatment and fell after discontinuance of the methyl testosterone. There appeared to be a second rise some 35 days after the drug was stopped.

DISCUSSION

It is clear from this study that methyl testosterone causes a profound decrease in the thyroxine-binding capacity of TBP in serum, presumably as a result of a decrease in the concentration of this protein. It seems likely that this is a primary effect of the hormone unrelated to any direct effects which testosterone might have on thyroid function and thyroxine metabolism. It is attractive, therefore, to consider the possibility that changes in thyroid function may be the result of this primary effect. According to this theory, one would expect the fall in TBP to result in an increased level of free thyroxine. The elevated free thyroxine might then result in an increased availability of thyroxine for metabolic degradation. Either as a direct effect of the ele-

TABLE IV

The influence of methyl testosterone on iodine metabolism: Radiothyroxine disappearance rate, organic iodine pool, and organic iodine degradation rate

Patient	Status	PBI* (μg./100 ml. serum)	Thyroxine disappearance rate (% per day)	Extra thyroidal organic iodine pool (μg. iodine/1.73 M.^{2})	Organic iodine degradation (μg. iodine degraded/day/1.73 M.^{2})
B. B.	Control	7.2	11	590	65
	Methyl testosterone (26)†	4.6	16	450	70
C. H.	Control	3.8	14	390	55
	Methyl testosterone (27)	2.2	22	390	85
	Control (159)	4.0	13	490	64
L. W.	Control	4.5	15	340	50
	Methyl testosterone (23)	3.6	18	300	54
	Control (30)	4.6	12	380	45
D. W.	Control	4.0	13	330	43
	Methyl testosterone (23)	3.6	19	330	61
	Control (130)	5.6	9.9	500	50

* Protein-bound iodine.
† The number in parentheses indicates days on testosterone, or off testosterone, at time of study.

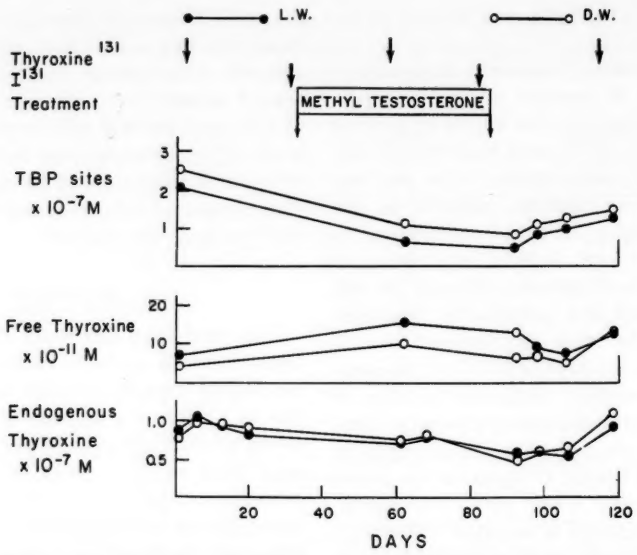

FIG. 2. EFFECT OF METHYL TESTOSTERONE ON THYROXINE-BINDING PROTEIN, FREE THYROXINE, AND PROTEIN-BOUND IODINE

The arrows after thyroxine^{131} and I^{131} represent, respectively, the times at which the labeled thyroxine was injected for the turnover studies and the time the iodide tracer studies were done.

vated free thyroxine or as a result of the increased thyroxine metabolism, pituitary secretion of thyrotropic hormone might then be suppressed, resulting in a decrease in the turnover and uptake of iodine by the thyroid gland. The combined effect of increased thyroxine degradation and decreased thyroxine secretion would lead to a fall in serum thyroxine and, ultimately, a new steady state in which the serum PBI is depressed, the free thyroxine level is normal, and thyroid iodide clearance, thyroid hormone secretion, and thyroxine degradation are normal.

In keeping with such a theory, this study revealed an increase in the free thyroxine level in serum, an increase in thyroxine degradation, and a fall in serum PBI in some or all of the subjects. It would appear that a new steady state had not yet been reached after four weeks of testosterone treatment, when TBP and thyroxine disappearance were determined, although after seven weeks it might have been (*vide infra*).

Several objections to the above interpretation should be considered.

1) The method of calculation of extrathyroidal organic iodine (by intercept), of degradation (by final slope), and of organic iodine degradation rate is at best approximate. Only if there are no changes in diffusion rates is the use of these values valid for comparison, even in the same patient.

2) The errors and assumptions involved in calculation of free thyroxine have been detailed previously and still obtain (11, 14). A further problem in the present study is the fact that with such low values for thyroxine-binding capacity of TBP, the accuracy of the free thyroxine value is strongly dependent on the precision with which the PBI has been measured. Thus, at low PBI concentrations, variation within the limits of error of the PBI method will produce large changes in the calculated free thyroxine. This may account for the erratic results of this calculation (Figure 2).

3) All the calculations assume a steady state situation. As indicated above, this probably did not obtain during the study. The use of methimazole during the thyroxine turnover studies introduces a further complicating factor since it

places the thyroid in an unsteady state. It secretes iodine but cannot bind it. In normal subjects there is a large thyroid iodine pool, so it may be presumed that a few weeks exposure to methimazole will not produce major changes in the thyroid.

4) The changes in thyroidal iodide clearance seen were not consistent and hence are difficult to interpret. It should be noted that all of these clearances and the uptake values were within the normal range (12, 15). Biological variation, alterations in iodine in diet, changes in secretion of adrenal steroids, goiterogens in food, and so forth, all contribute toward the difficulty of obtaining exactly reproducible data over a period of months for thyroid iodide clearance. A new steady state of thyroid activity may have been reached after 47 to 51 days of treatment with methyl testosterone. It was, unfortunately, not feasible to perform iodide tracers and thyroxine disappearance studies simultaneously. The serial determination of thyroidal iodide clearance in a large number of subjects before and during the administration of methyl testosterone would be desirable and probably necessary to determine precisely changes in this function during the unsteady state.

Other mechanisms for the effects noted during methyl testosterone administration could be mentioned. A direct effect on the pituitary to suppress its output of thyroid stimulating hormone is possible. The PBI would fall (as observed) but a marked fall in thyroid iodide clearance would also be expected (this was not observed). Furthermore, another mechanism to account for the increase in rate of degradation of thyroxine and fall in TBP would still be necessary. A direct effect on the thyroid to decrease secretion of thyroxine could explain the fall in PBI. If this mechanism were similar to the effect of iodine, there might be little change in thyroid iodide clearance. Nevertheless, the increased degradation of thyroxine and fall in TBP would still be unexplained. An effect similar to that produced by dinitrophenol, which increases the rate of metabolism of thyroxine and also suppresses the pituitary, is possible (16). Usually a marked fall in thyroid iodide clearance is seen, however, and again the fall in TBP is not explained.

Therefore, although the theory presented, which proposes that the effects of methyl testosterone result from a primary alteration in TBP, is not proven, it seems the most likely working hypothesis at present.

It is of considerable interest that estrogenic hormones and pregnancy, which have been shown to produce a rise in PBI in serum, have an effect on thyroxine-binding of TBP which is opposite to that found with methyl testosterone (11, 14, 17, 18). Detailed studies of other aspects of thyroid function during estrogen treatment in humans have not been reported.

The change in serum cholesterol cannot be directly inferred from this hypothesis, since, if anything, one would expect a fall in the level to correspond with the elevated free thyroxine level and utilization. An increase in serum cholesterol in male subjects with coronary artery disease and hypercholesterolemia when they were given methyl testosterone has been noted previously (19). No data are available to suggest whether this effect is upon cholesterol synthesis or excretion, or whether it is direct or indirect.

It is possible that the exacerbation of hyperthyroidism observed by Kinsell and co-workers in patients receiving methyl testosterone might be explained by the fall in TBP and the consequent elevation of the level of free thyroxine. Since testosterone propionate did not produce such an exacerbation, it should prove valuable to determine whether testosterone propionate will affect thyroxine-binding by TBP.

SUMMARY

A study of the influence of methyl testosterone on human thyroid function is presented. The drug induced a striking fall in thyroxine-binding capacity of the thyroxine-binding alpha globulin of serum in all patients, and a less striking but consistent increase in the fractional rate of thyroxine disappearance from blood. Other findings were a slight fall in the serum protein-bound iodine, an increase in circulating free thyroxine, and an increase in the amount of thyroxine degraded per day. There were inconstant effects on the thyroidal and renal clearance of iodide. The hypothesis is offered that a fall in concentration of thyroxine-binding protein in serum is responsible for the changes observed. A consistent rise in serum cholesterol was also noted during the administration of methyl testosterone.

ACKNOWLEDGMENTS

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RELATIONSHIP BETWEEN A RANGE OF TISSUE TEMPERATURE AND LOCAL OXYGEN UPTAKE IN THE HUMAN FOREARM. I. CHANGES OBSERVED UNDER RESTING CONDITIONS [1, 2]

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The advent of the use of hypothermia as an adjunct to surgery has revived interest in the effect of alterations of local temperature on tissue metabolism. In the early part of this century, several investigators (1, 2) performed *in vitro* studies and found that increasing the temperature of muscle slices raised their oxygen consumption, while an opposite effect was produced by decreasing the temperature. The Q_{10} of this relationship was generally found to be slightly above the value of two (2). However, one limitation of the work was that the absolute oxygen consumption was closely dependent upon the composition of the bath in which the tissue was suspended.

In more recent years a number of workers (3–5) have noted an increase in local circulation with exposure of a limb to high external temperatures and a decrease with cooling of the tissues. From such evidence it has been concluded that the alterations in blood flow produced by local heat and cold are due both to the direct action of these agents on the blood vessels and to changes in the metabolic needs of the tissues being supplied. However, it is apparent that data on the circulation alone are not sufficient for the determination of tissue oxygen utilization, and that for this purpose, the simultaneous measurement of the quantity of oxygen removed from the blood as it passes through the structures under study is likewise essential.

Studies on the effect of hypothermia on the whole organism have also contributed to the elucidation of the problem. In the rat (6) and in the dog (7, 8) this state is associated initially with a rise in oxygen consumption, corresponding to a stress reaction developed in the body, and then a fall as cooling is continued. If shivering, which is elicited in the first phase, is prevented by the use of barbiturates, the increase in oxygen consumption is no longer observed (7). In poikilothermic animals hypothermia produces only a reduction in oxygen consumption (9).

The purpose of this paper is to present the effects of local heating and cooling on the resting oxygen uptake of the tissues of the forearm.

METHOD

The investigation was performed on 17 healthy subjects between the ages of 19 and 51 years. Thirteen were males and four were females. In all, 27 experiments were done, their duration averaging six hours. In this period no food was served to the subject other than one or two small chocolate bars.

Three different procedures were carried out during each experiment: *1)* blood flow recordings on a six inch segment of forearm enclosed in a venous occlusion plethysmograph, according to the technique previously described [3] (10) (Figure 1); *2)* determinations of oxygen content of venous blood from deep or superficial veins of the forearm; and *3)* studies of muscle, subcutaneous tissue, skin, and rectal temperatures, using thermocouples. The temperature of the water in the plethysmograph (bath temperature) was first maintained at 45° C. and then lowered to 32° and finally to 17°,[4] and at each level all of the above procedures were done, together with blood pressure and pulse rate readings, obtained from the opposite extremity.

[1] This investigation was supported in part by Research Grant H-2568 from the National Institutes of Health, United States Public Health Service.

[2] This paper was presented at the International Cardiovascular Society meeting at San Francisco, Calif., on June 21, 1958.

[3] A modification of the method consisted of the use of an air reservoir with appropriate valves for the application of a venous collecting pressure. With such an arrangement a delay of somewhat more than a second occured in the build-up of the pressure in the cuff to the desired level, thereby practically eliminating artifacts in the blood flow record (Figure 1).

[4] Five pilot studies revealed that altering the sequence in which the forearm was exposed to the different bath temperatures did not materially affect the results.

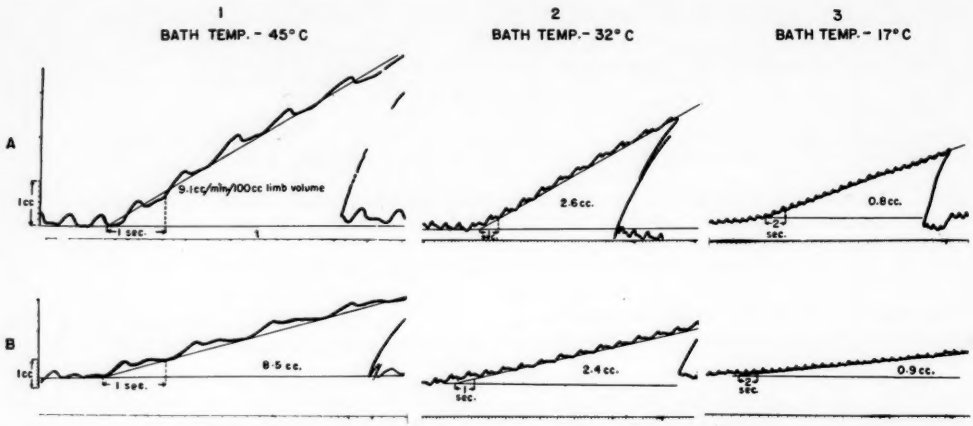

FIG. 1. RESTING BLOOD FLOW CURVES OBTAINED FROM THE FOREARM AT THREE DIFFERENT BATH TEMPERATURES, USING A VENOUS COLLECTING PRESSURE OF 60 MM. HG APPLIED TO THE ARM

Arterial occlusion pressure was maintained at the wrist before and during period of blood flow recording to prevent vascular changes in the hand from influencing readings obtained from the forearm. Calibration scale, in cubic centimeter divisions, to the left of the curves, was derived by introducing increments of 1 cc. of water into the plethysmograph with the forearm in position but with the circulation occluded. Blood flow, in cc. per minute per 100 cc. limb volume, was calculated on the basis of the height of the volume curve reached in first second of application of venous collecting pressure. Two seconds was frequently used in the case of bath temperature of 17° C. because of relative slowness of ascent of blood flow curve. A = Subject S. M.; B = Subject A. L.

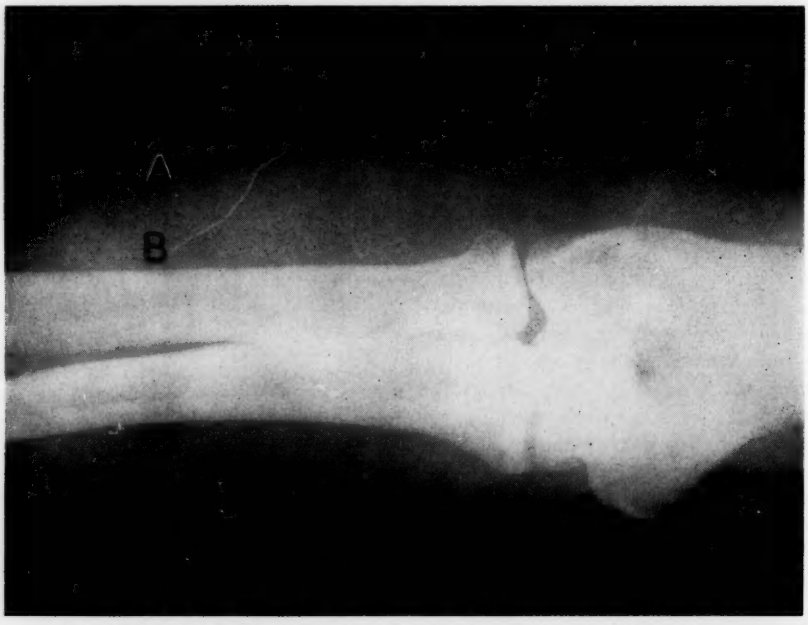

FIG. 2. SOFT TISSUE X-RAY SHOWING THERMOCOUPLES IN PLACE

A = subcutaneous tissue thermocouple. B = muscle thermocouple, with tip near the bone. Skin thermocouple, lying on the skin, is not clearly visualized.

The experiments were performed in a temperature-regulated room, with the temperature ranging between 22 and 25° C. and the humidity around 50 per cent. Psychic stimuli which could conceivably alter local circulation were kept at a minimum. The subject, dressed in a surgical gown, lay in the supine position during the entire experimental period. Care was taken to support the forearm at heart level in a constant position and angle with regard to the long axis of the body (11, 12).

Thermocouples were constructed of fine constantan and insulated copper wires completely enclosed in polyvinyl tubing (No. 442-T, Becton, Dickinson). The reference junction was immersed in a thermos jar filled with ice. The output of the thermocouples was recorded by means of a sensitive potentiometer calibrated to read directly in degrees centigrade.

Using aseptic technique and local anesthesia with 2 per cent procaine, a thin-walled No. 18 needle (3.7 cm. long) with obturator was inserted for a depth of approximately 3 cm. into the brachio-radialis muscle of the forearm. The obturator was then removed, a thermocouple was passed through the full length of the needle, and the latter was carefully withdrawn, leaving the thermocouple in place.[5] In a similar manner, a thermocouple was inserted into the subcutaneous tissue, parallel to the skin, for a distance of about 3 cm., while a third one was attached to the skin by rubber cement in the vicinity of the other two (Figures 2 and 3). A fourth modified thermocouple was inserted into the rectum for approximately 6 to 7 cm.

Blood samples for the determination of venous oxygen content were obtained from a vein in the antecubital space and analyzed according to the manometric method of Van Slyke. The technique used was as follows: First, under sterile precautions, a No. 18 thin-walled needle, pointed distally, was inserted into the vein. Then the free end of a sterile-silicone treated polyethylene catheter (PE 50, Clay-Adams), attached to a special metal coupler (PE 2625A, Clay-Adams), was threaded through the needle into the vessel for a distance of 5 to 8 cm., so that the tip lay in the mass of forearm subsequently enclosed by the plethysmograph. Following this the needle was withdrawn from the vein, leaving the tubing in place.[6] Blood samples were obtained according to the plan outlined in Figure 3.

No attempt was made to collect repeated arterial blood samples, but instead comparable data were derived by re-oxygenating venous blood and determining in duplicate total oxygen capacity. From this figure arterial oxygen content was calculated (13), on the basis that arterial blood is 98 per cent saturated with oxygen (14). Since all the subjects used in the study were normal, with no signs of cardiac or pulmonary pathology, such an assumption was considered valid. Furthermore, the data for the three bath temperatures were collected during the course of the same experiment, with the subject under constant environmental conditions and in approximately an unaltered physiologic state. The possibility, therefore, that a change in the oxygen content of arterial blood could have occurred in the course of the experimental period appeared unlikely.

The plan utilized in each experiment was as follows: First, a polyethylene tube was passed into a deep or superficial vein and connected to an infusion set (Figure 3). Then the various thermocouples were inserted into their respective sites and the wires directed toward the arm (Figure 3). Finally the forearm was passed into the plethysmograph, with the thermocouple wires being let out of the proximal opening parallel to the skin, and the openings were made water-tight according to the technique

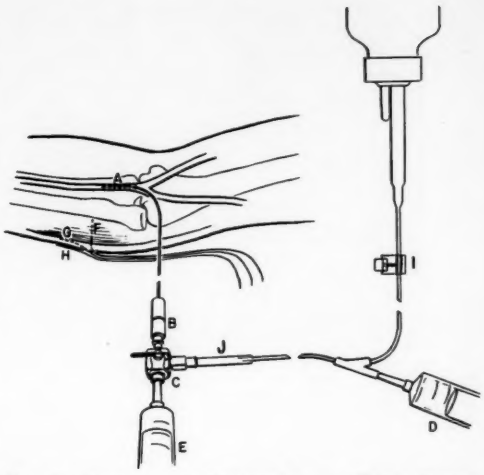

FIG. 3. STEPS IN THE COLLECTION OF A BLOOD SAMPLE

1) Apply and maintain arterial occlusion pressure at wrist for three to four minutes to prevent vascular changes in hand from influencing venous content of blood from forearm; *2*) close clamp I; *3*) aspirate fluid in tubing, using syringe D, until blood appears in side arm J; *4*) rotate handle of stopcock C 90 degrees so as to allow a drop of blood to drain away from free female opening; *5*) attach oiled syringe E, with dead space filled with a solution of heparin and sodium fluoride, to free opening and withdraw 3 cc. of blood in approximately 10 seconds; *6*) disconnect syringe, cover tip with metal cap and rotate rapidly to mix blood with anticoagulant; *7*) flush entire infusion system with saline containing heparin and then allow small quantity to enter vein continuously between blood sample collections. A = cannula in vein directed toward hand; B = special adapter for cannula. F, G, and H = muscle, subcutaneous tissue and skin thermocouples, respectively.

[5] In each instance the length of thermocouple in the tissues was determined at the end of the experiment. This was found to be an average of 3.5 cm., with a range of 1.8 cm. to 5.1 cm.

[6] In a number of instances, at the end of the experiment the cannulated vein was filled with radiopaque material through the polyethylene tube, and a soft-tissue X-ray was taken (Figure 4).

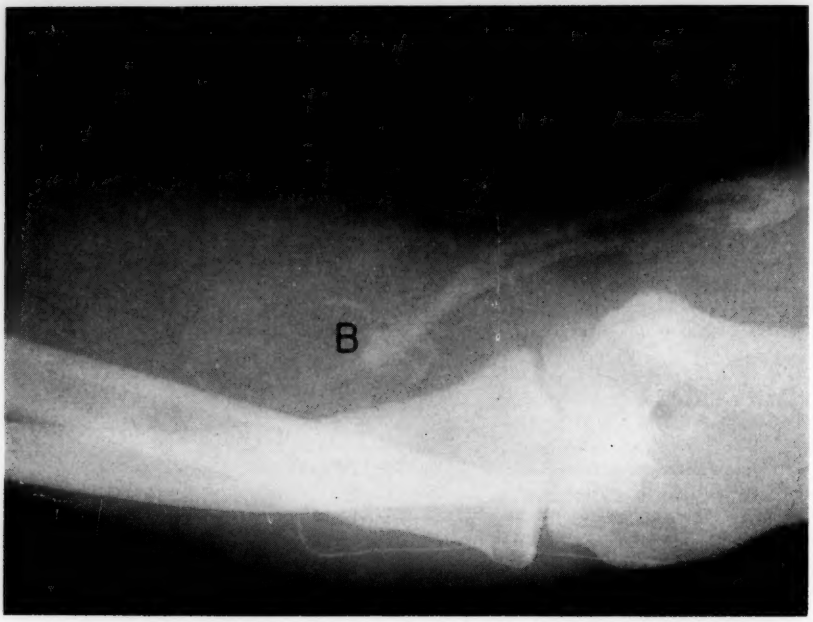

FIG. 4. CANNULATED VEIN OUTLINED WITH RADIOPAQUE SUBSTANCE

A = polyethylene cannula in vein; B = obstruction to distal movement of the material, probably because of the presence of a valve. Proximal portion of vein was filled with radiopaque substance. Thermocouples are seen in lower portion of the figure.

previously described (10). The next step was to fill the plethysmograph with water at a temperature of 45° C., which level was maintained for approximately 90 minutes until the various measurements became constant. Following this a series of five or six temperature and blood flow determinations was obtained, and, after a delay of two minutes, a blood sample was collected (15). The sequence of temperature and blood flow readings and blood sampling was repeated at least three or four times over the course of the next hour. In the first six experiments duplicate tests were run on each of the three to five blood samples, while in the remainder this was done only when the reading was not in accord with the other data obtained under the same conditions.

As soon as the above determinations were made, the plethysmograph was emptied of hot water and filled with water at a temperature of 32° C. The exact sequence described above was again carried out at this bath temperature, and then at a bath temperature of 17°, the latter condition being maintained through the use of a refrigeration system connected to a series of coils in the interior of the plethysmograph. With each of the two bath temperatures, the various measurements were obtained only after a period of 90 minutes had elapsed, in order to permit stabilization of the circulation at the new level.

Of the 27 experiments performed, in 17, blood samples were collected from a deep vein and in 10, from a superficial vein. However, it should be pointed out that at times it was difficult to identify with certainty the type of vessel cannulated. Furthermore, there was no way of determining whether an apparently superficial vein did not receive communicating vessels from the deep venous system and a deep vein, tributaries from the superficial venous system distal to its subcutaneous location.

RESULTS

From the collected data, resting oxygen uptake was calculated using the Fick principle (16). For the derivation of an average tissue temperature, the temperature readings at each of the three bath temperatures were weighted on the basis of the contribution of the tissue from which they were obtained to the total vascular mass of the forearm [combined skin and subcutaneous tissue, 17.4 per cent by weight; muscle, 82.6 per cent (17)].

Systemic changes

Pulse rates showed no consistent change at the different bath temperatures. In most instances the

readings were the same for the three experimental conditions, in some they were lowest at a bath temperature of 17° C., while in others they were lowest at a bath temperature of 45°. Blood pressure readings also showed no consistent or definite trend. At a bath temperature of 45° about one-half the subjects experienced a generalized sensation of warmth and sweating, due to reflex vasodilatation, while at bath temperatures of 32° and 17° no symptoms were noted. The average rectal temperature at the three bath temperatures varied between 37.3 and 37.7°.

Changes in blood oxygen

The mean derived oxygen content of arterial blood for all the subjects was 20.20 volumes per cent. The oxygen content of blood from deep veins showed a progressive decrease as the bath temperature was lowered (an average of 15.52, 12.78 and 10.62 volumes per cent at 45°, 32° and 17°, respectively), these alterations being reflected in a reverse trend in the case of the oxygen arteriovenous difference (an average of 4.79, 7.62 and 9.77 volumes per cent at 45°, 32° and 17°, respectively). A similar type of relationship was noted for the oxygen content of blood from superficial veins (an average of 16.18, 11.12 and 10.46 volumes per cent at 45°, 32° and 17°, respectively), and for the oxygen arteriovenous difference (an average of 3.73, 8.88 and 9.45 volumes per cent at 45°, 32° and 17°, respectively).

Changes in blood flow

For the group as a whole the average blood flow at a bath temperature of 45° C. was 7.3 cc. per minute per 100 cc. of limb volume; at a bath temperature of 32° it was 2.3 cc.; while at a bath temperature of 17° it was 0.7 cc.

Changes in oxygen uptake

Examination of Table I reveals that on the basis of blood obtained from deep veins, the calculated oxygen uptake at an average tissue temperature of 39.0° C. was 0.369 cc. per minute per 100 cc. limb volume, as compared with 0.199 cc. at an average tissue temperature of 35.4°, and 0.071 cc. at an average tissue temperature of 26.9°. When the data were subjected to statistical analysis, it was found that the probability that the difference between the results at average tissue temperatures of 39.0° and 35.4° arose simply by chance variation was less than 0.1 per cent ($p < 0.001$) (18). The probability that the difference between the figures obtained at average tissue temperatures of 35.4° and 26.9° was on the basis of sampling variation alone was also less than 0.1 per cent ($p < 0.001$).

Examination of Table II reveals that on the basis of blood obtained from superficial veins the oxygen uptake at an average tissue temperature of 38.9° C. was 0.261 cc. per minute per 100 cc. limb volume, as compared with 0.165 cc. at an average tissue temperature of 34.9°, and 0.070 cc. at an average tissue temperature of 26.7°. Statistical analysis of the data demonstrated that the probability that the difference between the results at average tissue temperatures of 38.9° and 34.9° arose simply by change variation was less than 1 per cent ($p < 0.01$). The probability that the difference between the figure obtained at average tissue temperatures of 34.9° and 26.7° was on the basis of sampling variation alone was also less than 1 per cent ($p < 0.01$).

DISCUSSION

There seems to be little question that in the present study raising the average temperature of the structures of the forearm by the surface application of heat produced an increase in oxygen uptake, while cold had an opposite effect (Tables I and II). This relationship held regardless of whether the venous blood used in the determination of the oxygen arteriovenous difference came from deep or superficial veins. Furthermore, the changes appeared to be due primarily to local alterations in the tissues, the systemic responses elicited by the different experimental conditions being minimal and inconstant.

It is necessary to point out, however, that at the high bath temperature the average oxygen uptake calculated on the basis of blood obtained from superficial tissues was less than that derived from oxygen content of blood draining deep tissues (0.261 cc. per minute per 100 cc. limb volume, as compared with 0.369 cc.). A possible explanation for such a difference is the following: Due to the proximity of the skin to the source of the heat, the cutaneous circulation was markedly en-

hanced, resulting in a large oxygen content of venous blood draining superficial tissues and a small oxygen arteriovenous difference (3.73 volumes per cent). At the same time, however, this augmented circulation was not reflected proportionately in the total blood flow figure, since the latter was the

TABLE I

Effect of different bath temperatures on resting oxygen uptake of the forearm based on blood samples from deep veins

Subject	O_2 capacity *cc./100 cc. blood*	Cal. art. O_2 *cc./100 cc. blood*	Venous O_2 *cc./100 cc. blood*	O_2 A. V. diff. *per cc. blood*	Blood flow *cc./min./100 cc. vol.*	O_2 uptake *cc./min./100 cc. vol.*	Temp. ° C. Skin	Subcutaneous tissue	Muscle	Average tissue	Rectal
Bath temperature—45° C.											
O. C.	21.76	21.32	17.57	0.0375	8.8	0.330	40.2	40.1	39.4	39.5	38.2
S. M.*	21.26	20.84	15.02	0.0487	9.4	0.544	41.0	39.0	37.8	38.2	37.5
H. H.	20.00	19.60	16.46	0.0314	8.9	0.279	41.5	40.1	37.6	38.2	37.1
S. T.*	19.77	19.37	15.84	0.0353	6.3	0.222	42.5	41.5	38.6	39.2	37.4
J. T.*	21.89	21.45	17.94	0.0351	6.7	0.235	40.8	40.6	38.3	38.7	37.5
A. K.†	21.57	21.14	17.90	0.0324	6.6	0.214	41.6	39.9	38.9	39.2	37.9
G. T.	19.64	19.25	15.29	0.0396	3.7	0.146	43.0	41.3	40.7	40.9	39.1
A. P.	21.37	20.94	11.11	0.0983	5.6	0.550	42.7	39.6	38.5	38.9	36.9
A. M.	18.00	17.64	11.91	0.0573	12.7	0.728	41.5	39.8	38.2	38.6	37.5
A. L.	20.49	20.08	13.90	0.0618	8.1	0.501	41.8	39.6	38.7	39.0	38.3
G. B.	23.06	22.60	16.82	0.0578	6.3	0.364	42.5	42.3	37.8	38.6	37.7
H. B.	20.98	20.56	16.54	0.0402	7.8	0.314	41.1	40.9	38.4	38.8	38.1
Mean	20.81	20.39	15.52	0.0479	7.6	0.369	41.7	40.4	38.6	39.0	37.7
Standard error				±0.0055	±0.6	±0.051					
Bath temperature—32° C.											
O. C.	21.76	21.32	12.82	0.0850	2.9	0.246	34.4	35.4	35.5	35.4	38.2
S. M.*	21.26	20.84	13.40	0.0744	2.8	0.208	33.2	34.1	35.7	35.2	37.0
H. H.	20.00	19.60	14.61	0.0499	4.0	0.200	33.3	34.0	35.9	35.5	36.7
S. T.*	19.77	19.37	14.02	0.0535	1.7	0.091	33.1	33.0	35.3	34.9	37.5
J. T.*	21.89	21.45	14.91	0.0654	1.9	0.124	32.5	33.2	35.6	35.1	37.3
A. K.†	21.57	21.14	13.22	0.0792	1.7	0.135	33.3	33.7	35.4	35.1	37.7
G. T.	19.64	19.25	12.29	0.0696	1.6	0.111	33.6	33.0	35.9	35.4	37.3
A. P.	21.37	20.94	9.74	0.1120	2.7	0.302	33.1	34.4	34.6	34.4	36.8
A. M.	18.00	17.64	10.18	0.0746	4.8	0.358	33.7	34.6	36.0	35.7	37.1
A. L.	20.49	20.08	10.12	0.0996	2.5	0.249	33.4	34.4	36.7	36.2	38.2
G. B.	23.06	22.60	15.66	0.0694	2.5	0.173	33.0	33.8	36.5	36.0	37.3
H. B.	20.98	20.56	12.40	0.0816	2.4	0.196	34.9	34.7	37.2	36.8	37.9
Mean	20.81	20.39	12.78	0.0762	2.6	0.199	33.4	34.0	35.8	35.4	37.4
Standard error				±0.0051	±0.3	±0.023					
Bath temperature—17° C.											
O. C.	21.76	21.32	12.75	0.0857	1.0	0.086	23.9	21.6	26.3	25.7	38.1
S. M.*	21.26	20.84	11.62	0.0922	0.8	0.074	18.7	21.1	27.5	26.2	36.9
H. H.	20.00	19.60	12.59	0.0701	0.7	0.049	18.3	19.7	29.8	27.9	36.6
S. T.*	19.77	19.37	12.55	0.0682	0.6	0.041	24.3	24.1	28.3	27.6	37.3
J. T.*	21.89	21.45	11.48	0.0997	0.5	0.050	18.3	18.3	27.1	25.6	37.1
A. K.†	21.57	21.14	11.30	0.0984	0.7	0.069	19.1	21.0	26.0	24.9	37.6
G. T.	19.64	19.25	9.37	0.0988	0.6	0.059	21.8	22.8	29.8	28.5	37.3
A. P.	21.37	20.94	9.45	0.1149	1.0	0.115	18.3	20.0	25.5	24.4	36.6
A. M.	18.00	17.64	6.96	0.1068	0.8	0.085	19.6	21.2	27.5	26.3	36.8
A. L.	20.49	20.08	7.99	0.1209	0.7	0.085	19.5	21.4	27.3	26.1	38.1
G. B.	23.06	22.60	10.69	0.1191	0.6	0.071	18.5	19.6	32.9	30.5	37.3
H. B.	20.98	20.56	10.76	0.0980	0.7	0.069	20.4	20.0	31.8	29.8	37.7
Mean	20.81	20.39	10.62	0.0977	0.7	0.071	20.1	20.9	28.3	26.9	37.3
Standard error				±0.0049	±0.1	±0.006					

* Average of two experiments.
† Average of three experiments.

TABLE II

Effect of different bath temperatures on resting oxygen uptake of the forearm based on blood samples from superficial veins

Subject	Bath temperature—45° C.		Bath temperature—32° C.		Bath temperature—17° C.	
	Average tissue temp.	O_2 uptake *cc./min./ 100 cc.vol.*	Average tissue temp.	O_2 uptake *cc./min./ 100 cc. vol.*	Average tissue temp.	O_2 uptake *cc./min./ 100 cc. vol.*
O. C.	39.5	0.328	35.4	0.281	25.7	0.095
S. M.*	38.0	0.383	35.0	0.159	25.8	0.060
S. L.	39.4	0.115	33.7	0.101	24.3	0.050
A. P.	38.9	0.279	34.4	0.225	24.4	0.105
J. J.	38.9	0.117	36.1	0.055	29.9	0.040
D. A.*	39.0	0.345	34.5	0.189	27.0	0.107
B. K.	38.6	0.391	35.3	0.200	27.1	0.039
M. B.	39.1	0.131	35.1	0.109	29.1	0.061
Mean	38.9	0.261	34.9	0.165	26.7	0.070

* Average of two experiments.

resultant of vascular changes not only in the superficial tissues, but also in muscles, in which the vasodilating effect of the surface heat was not as great. It would be expected, therefore, that an oxygen uptake figure derived from an oxygen arteriovenous difference which was too low to represent changes in the total vascular tissue would be smaller than the true value.

In regard to the calculation of oxygen uptake based on blood draining deep tissues, the situation was reversed. Under these circumstances a higher oxygen arteriovenous difference was obtained (4.79 volumes per cent), due to the relatively slower circulation in the muscles, while at the same time the figure for total blood flow more than reflected the vascular changes in this tissue because of the contribution of the markedly enhanced cutaneous circulation. Hence, an oxygen uptake figure derived from such data would most likely be greater than the true value.

It would seem, then, that in the case of a high bath temperature, the reported average reading for oxygen uptake based on blood from superficial tissues was probably too low, while that based on blood from deep tissues was probably too high, the true value falling somewhere between the two.

It is of interest to consider possible mechanisms responsible for the differences in oxygen uptake observed under the various experimental conditions. At the highest tissue temperature the increase in oxygen uptake appeared to be satisfied primarily by an augmentation in blood flow, with the oxygen arteriovenous difference actually being smaller than at the two lower tissue temperatures. The latter finding would appear to indicate that the resulting increase in local circulation was greater than adequate to satisfy the higher metabolic needs, and, as a consequence, less than the normal quantity of oxygen was removed from each cc. of blood as it passed rapidly through the tissues. It is understandable why the described changes occurred, since it is generally accepted that heat is a very potent vasodilating agent.

At the low experimental tissue temperature, a reverse situation existed. There was a marked reduction in blood flow, together with a greater removal of oxygen from each cc. of blood. The latter change occurred despite the fact that the rate of oxygen dissociation is reduced at a lower tissue temperature.[7] The finding of an increase in oxygen arteriovenous difference would appear to indicate that the reduction in blood flow was of greater magnitude than warranted on the basis of diminished oxygen uptake, and, as a result, more than the normal quantity of oxygen had to be removed from each cc. of blood as it passed slowly through the tissues. The fact that cold is a very potent vasoconstricting stimulus explains the type of reaction noted at the low tissue temperature.

It would appear, therefore, that the changes in oxygen uptake produced in the tissues of the forearm by the topical application of heat and cold were dependent primarily upon alterations in local blood flow, with the mechanism of removal of oxy-

[7] However, it is necessary to point out that an increased local acidity may be produced at low tissue temperatures because of an inadequate blood flow, and that such a trend would facilitate oxygen dissociation.

gen from the blood playing a secondary and dependent role.

SUMMARY AND CONCLUSIONS

1) The effect of altering the local external temperature on the resting oxygen uptake of the tissues of the forearm was investigated in a series of 27 experiments performed on 17 normal subjects.

2) Oxygen uptake was calculated on the basis of blood flow determinations, using the venous occlusion plethysmographic method, and of studies of local oxygen arteriovenous difference.

3) On the basis of blood obtained from deep tissues, the calculation of oxygen uptake at an average tissue temperature of 39.0° C. (produced by a bath temperature of 45° C.) was 0.369 cc. per minute per 100 cc. limb volume; at a tissue temperature of 35.4° C. (produced by a bath temperature of 32° C.) it fell to 0.199 cc.; while at a tissue temperature of 26.9° C. (produced by a bath temperature of 17° C.), it was only 0.071 cc. The differences at the three levels of tissue temperature were found to be statistically significant.

4) On the basis of blood draining superficial tissues, the calculation of oxygen uptake at an average tissue temperature of 38.9° C. was 0.261 cc. per minute per 100 cc. limb volume, as compared with 0.165 cc. at an average tissue temperature of 34.9°, and 0.070 cc. at an average tissue temperature of 26.7°. These differences were also found to be statistically significant.

It was concluded that raising the local temperature of tissues *in vivo* definitely increased their resting oxygen uptake, while lowering tissue temperature produced a marked reduction in this figure. These results were considered to be in line with the *in vitro* studies on tissue strips and the hypothermia experiments on the whole organism.

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RELATIONSHIP BETWEEN A RANGE OF TISSUE TEMPERATURE AND LOCAL OXYGEN UPTAKE IN THE HUMAN FOREARM. II. CHANGES OBSERVED AFTER ARTERIAL OCCLUSION, IN THE PERIOD OF REACTIVE HYPEREMIA[1,2]

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It is generally accepted that the magnitude of the hyperemic response which follows deprivation of local circulation is related to the metabolic needs of the involved tissues (1–3). However, as has already been pointed out (4), conclusions regarding oxygen consumption cannot be based on blood flow measurements alone.

In the present investigation, therefore, it was decided to study the excess oxygen uptake of the tissues of the forearm (exclusive of bone) after a period of arterial occlusion, using the Fick principle. Furthermore, it was considered of interest to determine whether or not an experimentally produced alteration in tissue temperature influenced the magnitude of the oxygen repayment during reactive hyperemia in the same manner as has been reported in the case of oxygen uptake of resting tissues (4).

METHOD

Twenty-seven experiments were performed on 23 healthy subjects between the ages of 18 and 52 years. Sixteen were males and seven were females. The duration of an experiment averaged six hours, during which time food intake was restricted to one or two small chocolate bars.

Utilizing the same techniques and environmental conditions described in the previous paper (4), the following measurements were made: blood flow, oxygen content of venous blood and muscle,[3] subcutaneous tissue and skin temperatures, all obtained from the upper two-thirds of the forearm. The temperature of the water in the plethysmograph (bath temperature) was first maintained at 45° C. and then lowered to 17°, and under each of these experimental conditions all of the above measurements were taken, together with blood pressure and pulse rate readings, obtained from the opposite limb, and determinations of rectal temperature. For reasons discussed below, attempts were made to cannulate a vein in the antecubital fossa which had no obvious superficial tributaries and which appeared to drain deep tissues primarily. However, as already pointed out (4), it was not always possible to achieve this aim.

Plan of experiment. After tissue temperature readings were obtained under resting conditions and with air in the plethysmograph (at an average temperature of 29.3° C.), the machine was filled with water at a temperature of 45° C. Following a period of more than 90 minutes of exposure of the forearm to the heat, control values for blood flow, venous oxygen content and tissue temperatures were collected. Then compressed air at an arterial pressure was permitted to enter the 6 cm. wide pneumatic cuff on the arm (ordinarily used for the application of a collecting pressure in the production of a blood flow reading) and this level was maintained for five minutes.

Immediately after the sudden release of the arterial occlusion pressure on the arm, blood flow readings (Figure 1), venous blood samples and temperature recordings were obtained at rapid intervals, generally four to five of each being collected in the first minute of reactive hyperemia, two to three in the second, and four spaced over the next four to eight minutes. In every instance care was taken not to draw a blood sample at the same time that a blood flow was being recorded.[4] It was felt that removal of blood from the segment of forearm in the plethysmograph during such a period might detract from the rate of enlargement of the limb produced by the venous collecting pressure and thus decrease the blood

[1] This investigation was supported in part by Research Grant H-2568 from the National Institutes of Health, United States Public Health Service.

[2] This paper was presented at the International Cardiovascular Society meeting at San Francisco, Calif., on June 21, 1958.

[3] The muscle thermocouple was inserted perpendicularly to the skin into the upper portion of the forearm for an average distance of 4.2 cm. (range of 3.0 to 6.0 cm.).

[4] Since the initial blood flow readings were obtained by applying a venous occlusion pressure for no more than three seconds, such a precaution offered no real difficulty in obtaining an adequate number of blood samples.

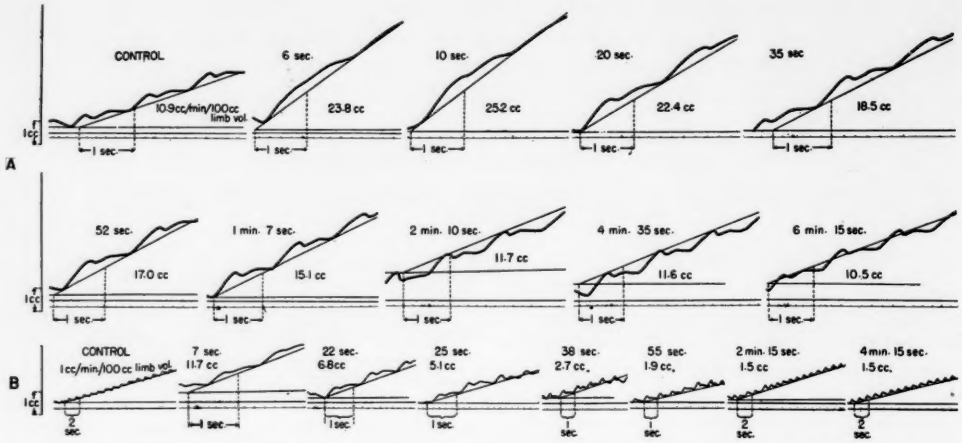

FIG. 1. FOREARM BLOOD FLOW CURVES OBTAINED ON SUBJECT C. M. BEFORE AND AFTER A FIVE MINUTE PERIOD OF ARTERIAL OCCLUSION

A = control and postocclusion readings collected at a bath temperature of 45° C. B = control and postocclusion readings collected at a bath temperature of 17° C. The calculated blood flow, in cc. per minute per 100 cc. limb volume, and the time in the period of reactive hyperemia are indicated for each determination. Calibration scale, in cc. divisions, is to the left of the curve. An arterial occlusion pressure was maintained at the wrist during blood flow recordings.

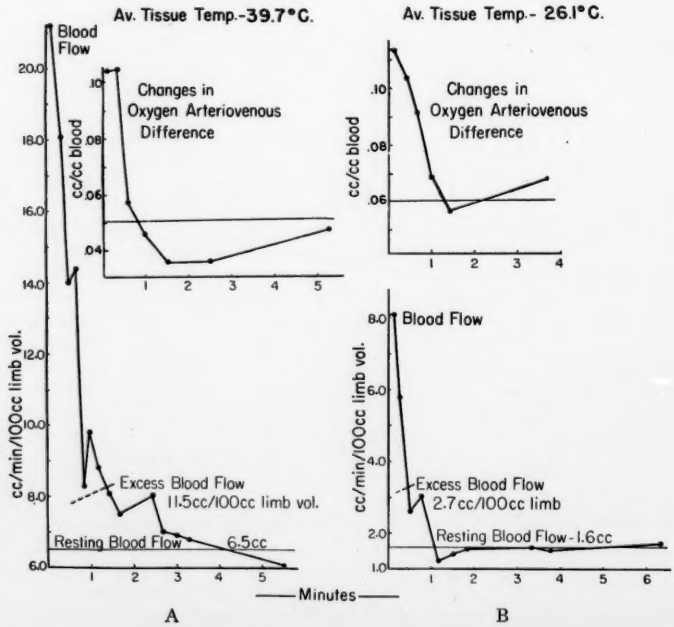

FIG. 2. GRAPHS OF BLOOD FLOW AND OXYGEN ARTERIOVENOUS DIFFERENCES OBTAINED DURING REACTIVE HYPEREMIA ON SUBJECT J. S.

A = bath temperature of 45° C. B = bath temperature of 17° C.

flow reading. The possibility that the collecting pressure might raise the venous oxygen in the sample of blood was not seriously considered, for the period of application utilized early in reactive hyperemia was only approximately three seconds and, therefore, not long enough to have any effect (5).

Throughout the experiment the venous collecting pressure was 60 mm. Hg, despite the possible objection, recently expressed (6), that this level might be too high for the determination of the initial blood flow obtained four seconds after the onset of reactive hyperemia. With the method used for the application of the venous collecting pressure, there was a delay of more than one second in the build-up in the cuff to 60 mm. Hg, and, consequently, it was felt that the height of pressure initially was not great enough to interfere with arterial inflow. This view was supported by the results obtained in a group of six subjects in whom first a venous collecting pressure of 60 mm. Hg was used and then, in another period, a pressure of 40 mm. Hg. Both under resting conditions and during reactive hyperemia at high and low bath temperatures, the blood flow readings were substantially the same for the two procedures, even early in the period of re-establishment of circulation.

After the various readings had returned to the pre-occlusion baseline, the bath temperature was lowered to 17° C. and maintained at this level for approximately 90 minutes before control sets of readings were collected. When this was accomplished the arterial circulation was again occluded for five minutes. On release of the pressure data were collected at rapid intervals, as in the case of a bath temperature of 45° C.

RESULTS

From the readings of blood flow and derived oxygen arteriovenous difference obtained at the two bath temperatures, oxygen uptake was calculated both for the resting state and for the period of reactive hyperemia, using the Fick principle (7). The predicted oxygen debt developed during the period of arterial occlusion was determined by multiplying the resting oxygen uptake by five minutes (the duration of application of the arterial occlusion pressure).

Three different graphs were constructed for each bath temperature, on the basis of changes in *1*) blood flow, *2*) oxygen arteriovenous difference, and *3*) oxygen uptake, respectively, observed in the period of reactive hyperemia (Figures 2 to 5). In each instance the figures were plotted against time in minutes, while a baseline was derived generally by averaging three to five control readings collected before arterial occlusion with a similar number obtained after reactive hyperemia had subsided. In the case of the blood

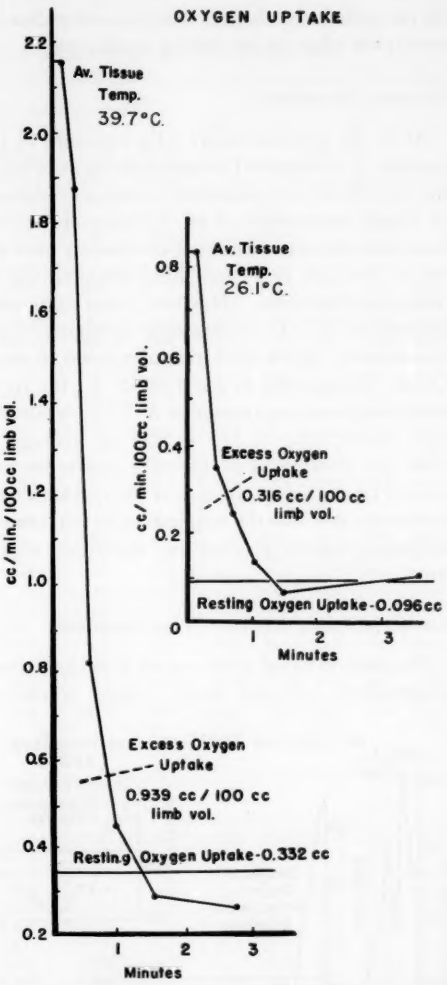

FIG. 3. GRAPHS OF OXYGEN UPTAKE OBTAINED DURING REACTIVE HYPEREMIA ON SUBJECT J. S.

Lower curve = bath temperature of 45° C. Upper curve = bath temperature of 17° C. Graphs are based on blood flow and oxygen arteriovenous differences as depicted in Figure 2.

flow and oxygen uptake graphs, the area under the curve and above the baseline was then determined by means of a planimeter and expressed in proper units by multiplying the result by an appropriate factor (Figures 2 to 5). The product was considered to represent the response produced solely by the period of arterial occlusion, since it

did not include the changes which would ordinarily have taken place under resting conditions.

Systemic alterations

As in the previous study (4), exposure of the forearm to extremes of temperature did not cause any significant or consistent systemic reactions. At a bath temperature of 45° C. some of the subjects experienced a sensation of warmth over the rest of the body and generalized sweating due to reflex vasodilatation. However, at a bath temperature of 17° C. no systemic symptoms were experienced. With the forearm exposed to room air and subsequently to water at 45° C., the rectal temperature was an average of 38.1° C., while at a bath temperature of 17° it was an average of 37.8° C. Resting blood pressure and pulse rate showed no consistent changes at the two bath temperatures; nor did the application of an arterial occlusion pressure produce any significant alterations.

Results obtained under resting conditions

The data on blood flow, venous blood and tissue temperatures collected under resting conditions

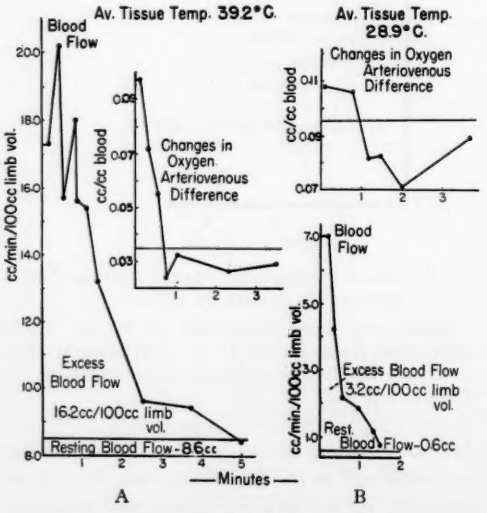

FIG. 4. GRAPHS OF BLOOD FLOW AND OXYGEN ARTERIOVENOUS DIFFERENCES OBTAINED DURING REACTIVE HYPEREMIA ON SUBJECT H. M.

A = bath temperature of 45° C. B = bath temperature of 17° C.

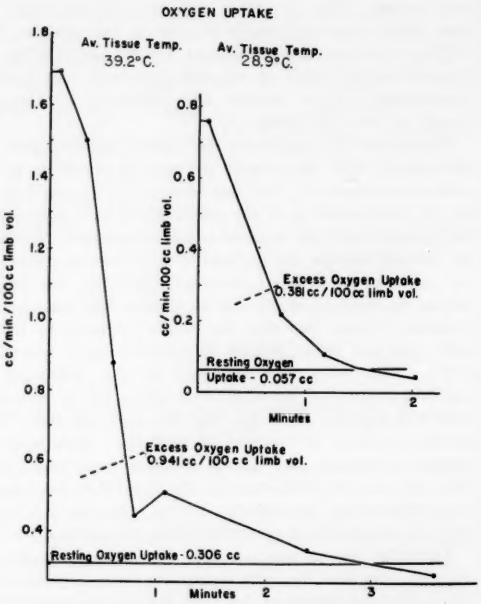
OXYGEN UPTAKE
Av. Tissue Temp. 39.2°C.
Av. Tissue Temp. 28.9°C.
cc/min./100 cc limb vol.
Excess Oxygen Uptake 0.381 cc/100 cc limb vol.
Resting Oxygen Uptake - 0.057 cc
Minutes
Excess Oxygen Uptake 0.941 cc/100 cc limb vol.
Resting Oxygen Uptake - 0.306 cc
Minutes

FIG. 5. GRAPHS OF OXYGEN UPTAKE OBTAINED DURING REACTIVE HYPEREMIA ON SUBJECT H. M.

Lower curve = bath temperature of 45° C. Upper curve = bath temperature of 17° C. Graphs are based on blood flow and oxygen arteriovenous differences as depicted in Figure 4.

are included in Table I. It will be noted that the average values for these readings at the two bath temperatures, as well as the calculations of oxygen uptake, were for the most part the same as those reported in a previous paper (4). The average temperature readings obtained in room air were: skin, 35.4° C.; subcutaneous tissue, 35.3°; and muscle, 36.2°. The average tissue temperature under these circumstances was 36.0°.

Blood flow responses during reactive hyperemia

The blood flow curves obtained at the two bath temperatures after removal of the arterial occlusion pressure were similar in general contour (Figures 2 and 4). Under both experimental conditions the peak of the response occurred soon after the onset of reactive hyperemia, although being noted a little earlier at the lower bath temperature (an average of 9 seconds, as compared with 19 seconds for the higher bath temperature). Thereafter the curve fell either gradually or

TABLE I

Effect of different bath temperatures on oxygen uptake of the forearm during reactive hyperemia

Subject	O_2 capacity *cc./100 cc. blood*	Calculated art. O_2 *cc./100 cc. blood*	Resting venous O_2 *cc./100 cc. blood*	Resting O_2 A. V. diff. *cc./cc. blood*	Resting blood flow *cc./min./100 cc. limb vol.*	Resting O_2 uptake *cc./min./100 cc. limb vol.*	Excess O_2 uptake during reactive hyperemia *cc./100 cc. limb vol.*	Predicted excess O_2 uptake during reactive hyperemia *cc./100 cc. limb vol.*	Excess blood flow during reactive hyperemia *cc./100 cc. limb vol.*	Temp. °C. Skin	Subcutaneous tissue	Muscle	Average tissue	Rectal
Bath temperature—45° C.														
M. M.	17.38	17.03	12.99	0.0404	8.4	0.343	0.871	1.715	7.8	40.8	40.2	39.2	39.4	38.3
H. R.*	20.53	20.12	16.15	0.0397	7.9	0.314	0.522	1.570	5.9	42.0	41.1	38.6	39.1	38.1
B. M.	19.63	19.24	16.45	0.0279	9.6	0.268	0.660	1.340	6.0	41.3	40.8	39.5	39.8	38.4
A. L.	20.49	20.08	14.20	0.0588	8.8	0.517	0.862	2.585	15.6	41.8	39.6	38.7	39.0	38.3
S. M.	21.16	20.74	16.38	0.0436	10.0	0.436	0.991	2.180	10.0	41.3	38.7	38.0	38.3	37.7
E. G.	20.31	19.90	13.94	0.0596	8.0	0.477	0.731	2.385	8.2	43.9	42.9	39.7	40.3	38.2
G. B.	21.98	21.54	17.32	0.0422	9.2	0.388	1.820	1.940	14.5	42.6	41.4	38.5	39.1	38.3
H. M.	20.99	20.57	17.01	0.0356	8.6	0.306	0.941	1.530	16.2	41.5	40.7	38.8	39.2	38.5
A. R.	18.85	18.47	14.18	0.0429	4.8	0.206	1.226	1.030	12.7	42.5	41.8	41.2	41.4	38.6
J. B.*	17.86	17.50	11.22	0.0628	8.6	0.540	0.460	2.700	9.1	43.0	42.1	39.8	40.3	38.4
A. K.	20.18	19.78	12.39	0.0739	9.6	0.709	0.283	3.545	11.9	43.0	42.5	39.9	40.4	38.7
J. S.	20.73	20.31	15.20	0.0511	6.5	0.332	0.939	1.660	11.5	42.9	41.5	39.2	39.7	38.2
E. W.	17.29	16.94	14.23	0.0271	8.2	0.222	0.773	1.110	7.6	42.8	41.0	38.1	38.8	37.6
M. S.	19.32	18.93	14.82	0.0411	10.4	0.427	0.821	2.135	9.6	42.0	40.5	38.8	39.2	37.9
E. P.	20.42	20.01	15.10	0.0491	8.5	0.417	0.532	2.085	10.7	41.7	42.2	39.1	39.6	38.5
A. M.	18.00	17.64	11.94	0.0576	11.7	0.667	0.613	3.335	7.5	41.4	39.8	38.2	38.6	37.4
H. H.*	20.27	19.86	15.76	0.0410	8.6	0.353	0.968	1.765	13.4	41.3	40.4	37.9	38.4	37.4
M. R.*	21.19	20.77	16.19	0.0458	8.2	0.376	0.733	1.880	13.3	41.8	40.9	39.1	39.5	38.0
D. A.	19.86	19.46	15.96	0.0350	7.0	0.245	0.803	1.225	5.0	41.4	41.6	38.5	39.0	38.0
C. M.	16.34	16.01	9.55	0.0645	11.1	0.716	0.397	3.580	16.7	43.2	41.6	38.2	38.9	38.8
W. B.	17.34	16.99	12.75	0.0424	9.2	0.390	0.507	1.950	6.5	40.6	41.4	38.7	39.1	38.0
D. B.	23.06	22.60	17.01	0.0569	6.1	0.347	0.997	1.735	3.7	42.5	42.3	37.8	38.6	37.6
M. E.	17.26	16.91	14.74	0.0217	13.8	0.299	1.042	1.495	13.7	42.3	40.5	39.3	39.7	38.4
Mean	19.58	19.19	14.59	0.0461	8.8	0.404	0.804	2.021	10.3	42.1	41.1	38.9	39.3	38.1
Standard error				±0.0027	±0.4	±0.030	±0.067							
Bath temperature—17° C.														
M. M.	17.38	17.03	9.48	0.0754	0.8	0.060	0.259	0.300	2.6	20.2	20.6	26.3	25.3	38.3
H. R.*	20.53	20.12	13.26	0.0686	1.0	0.069	0.281	0.345	4.5	20.2	20.4	31.0	29.1	38.1
B. M.	19.63	19.24	13.73	0.0551	1.5	0.083	0.648	0.415	8.2	19.1	21.5	28.2	26.8	38.2
A. L.	20.49	20.08	9.24	0.1084	0.8	0.087	0.241	0.435	1.8	19.6	21.4	27.3	26.1	38.1
S. M.	21.16	20.74	12.35	0.0838	0.9	0.075	0.410	0.375	3.2	17.3	21.3	25.3	24.2	37.1
E. G.	20.31	19.90	9.79	0.1011	0.7	0.071	0.422	0.355	2.9	18.3	18.6	25.8	24.5	38.0
G. B.	21.98	21.54	19.22	0.0232	2.3	0.053	0.825	0.265	12.7	20.5	21.6	32.0	30.1	36.8
H. M.	20.99	20.57	11.07	0.0950	0.6	0.057	0.381	0.285	3.2	18.7	26.9	30.2	28.9	38.4
A. R.	18.85	18.47	8.45	0.1002	0.7	0.070	0.481	0.350	5.1	19.7	20.1	28.8	27.2	37.7
J. B.*	17.86	17.50	9.36	0.0814	1.4	0.114	0.644	0.570	6.3	18.8	19.9	28.1	26.6	38.1
A. K.	20.18	19.78	12.05	0.0773	1.2	0.093	0.304	0.465	4.5	19.4	19.8	26.4	25.2	38.4
J. S.	20.73	20.31	14.18	0.0601	1.6	0.096	0.316	0.480	2.7	18.7	20.0	27.5	26.1	38.0
E. W.	17.29	16.94	13.83	0.0311	0.9	0.028	0.301	0.140	2.6	18.0	20.8	25.7	24.6	37.6
M. S.	19.32	18.93	9.07	0.0985	0.6	0.059	0.341	0.295	2.6	18.9	20.2	25.7	24.6	37.7
E. P.	20.42	20.01	10.75	0.0921	0.5	0.046	0.222	0.230	2.1	17.1	18.6	26.4	24.9	38.2
A. M.	18.00	17.64	8.97	0.0867	0.8	0.069	0.480	0.345	3.6	19.6	21.2	27.5	26.3	36.8
H. H.*	20.27	19.86	14.15	0.0571	0.7	0.040	0.502	0.200	4.6	17.1	18.8	28.1	26.3	37.0
M. R.*	21.19	20.77	14.15	0.0662	1.1	0.073	0.339	0.365	3.1	18.6	20.8	26.4	25.2	38.0
D. A.	19.86	19.46	10.43	0.0903	0.7	0.063	0.495	0.315	5.1	20.0	19.3	29.8	28.0	37.8
C. M.	16.34	16.01	11.09	0.0492	1.2	0.059	0.385	0.295	6.0	19.3	22.0	30.7	28.9	38.4
W. B.	17.34	16.99	9.61	0.0738	0.9	0.066	0.247	0.330	2.3	21.8	20.7	30.3	28.7	36.6
D. B.	23.06	22.60	10.18	0.1242	0.7	0.087	0.611	0.435	4.0	18.5	19.6	32.9	30.5	37.3
M. E.	17.26	16.91	11.02	0.0589	1.4	0.082	0.286	0.410	5.3	18.2	20.7	30.5	28.6	38.1
Mean	19.58	19.19	11.54	0.0764	1.0	0.070	0.410	0.348	4.3	19.0	20.6	28.3	26.8	37.8
Standard error				±0.0050	±0.1	±0.004	±0.029							

* Average of two experiments.

abruptly until the resting blood flow baseline was reached or crossed. This took place an average of 211 and 153 seconds after re-establishment of the circulation, for the high and low bath temperatures, respectively.

In every instance the maximum height the blood flow curve attained was much greater at a bath temperature of 45° C. than at 17° [an average of 18.4 cc. per minute per 100 cc. limb volume, as compared with 8.0 cc. (Figures 2 and 4)]. However, when the actual maximum increase in excess of the resting level was calculated, this type of relationship was not as constant, being found in 20 experiments, while in the remaining 7 the figure was greater at the low bath temperature. Nevertheless, the average maximum rise in excess of the resting level for the group as a whole was still larger at the high bath temperature (an average of 9.8 cc. per minute per 100 cc. limb volume, as compared with 7.0 cc.).

The increase in blood flow in excess of the resting level during the period of reactive hyperemia was greater at the high bath temperature in all instances but four, while in the latter the reverse was true. For the group as a whole, the average excess blood flow at a bath temperature of 45° C. was 10.3 cc. per 100 cc. limb volume, as compared with 4.3 cc. at a bath temperature of 17° (Table I). Statistical analysis of the data revealed that this difference was very highly significant, with the probability that it rose simply by chance variation being less than 0.1 per cent ($p < 0.001$) (8).

Changes in oxygen arteriovenous difference during reactive hyperemia

The alterations in this figure following release of the arterial occlusion pressure showed no consistent pattern, as contrasted with the fairly uniform type of change observed in the blood flow response. At the high bath temperature in all instances but one the initial reading, obtained within four to six seconds after re-establishment of the circulation, demonstrated an increase in the oxygen arteriovenous difference that was greater than the subsequent determinations (Figures 2 and 4); in the one exception the first point was located below the baseline and then there was a rise above it. At the low bath temperature, in 21 cases the initial reading showed the greatest increase in oxygen arteriovenous difference (Figure 2); in 3 the second reading was the highest; while in 3 all the points fell on either side of the baseline. A considerable range in the magnitude of the initial peak reading was observed at the two bath temperatures. In 19 cases the height of the rise was greater at the high than at the low bath temperature; in 3, the reverse was true; and in 5, the figures were approximately the same under the two different environmental conditions.

In most instances at both the high and low bath temperature there was a rapid drop of the curve from the peak reading so that the baseline was reached within the first minute of the period of reactive hyperemia. At the high bath temperature, in 16 instances this level was not crossed, while in the remaining 11 the curve fell below it, remaining there for from one to three minutes (Figures 2 and 4). At the low bath temperature there was a drop below the baseline in 21 experiments (Figures 2 and 4), while in the remaining 6 all the readings remained above the baseline. Of the cases already described, in four instances at the high bath temperature and in six at the low bath temperature, alterations in oxygen arteriovenous difference showed little tendency to vary from the baseline.

Changes in oxygen uptake during reactive hyperemia

As in the case of blood flow curves, the oxygen uptake graphs obtained at the two bath temperatures after release of the arterial occlusion pressure demonstrated certain similarities. Under both experimental conditions the peak of the response was noted within 3 to 15 seconds after re-establishment of the circulation, and in most instances this was followed by either a gradual or abrupt drop in the direction of the baseline (Figures 3 and 5). In four cases at the high bath temperature and in two at the low, a secondary and minor peak was observed 10 to 15 seconds after the initial and major rise. At the high bath temperature, in eight instances the curve fell below the baseline and remained there for from one to four minutes until it began to ascend again. This type of change was present only twice in the case of the low bath temperature.

Comparison of the oxygen uptake curves re-

vealed that in every instance the peak was much higher at a bath temperature of 45° C. than at 17° (an average of 1.745 cc. per minute per 100 cc. limb volume, as compared with 0.838 cc.). When the maximum increase in excess of the resting level was calculated, the above relationship was now observed in 22 cases, while in 5 the reading was greater at the lower bath temperature. The average rise above the baseline was 1.340 cc. per minute per 100 cc. limb volume at the high bath temperature and 0.769 cc. at the low bath temperature. The average period of time during which the oxygen uptake was still elevated above the resting level was longer at the high than the low bath temperature (187 as compared with 147 seconds).

The average excess oxygen uptake during reactive hyperemia at the high bath temperature was 0.804 cc. per 100 cc. of limb volume, as compared with 0.410 cc. at the low bath temperature (Table I). The data were analyzed statistically and it was found that this difference was very highly significant, with the probability that it rose simply by chance variation being less than 0.1 per cent ($p < 0.001$) (8). In only one instance (J. B.) was the excess oxygen uptake during reactive hyperemia higher at 17° than at 45°.

Comparison of actual oxygen uptake during reactive hyperemia with predicted oxygen debt for period of arterial occlusion

Examination of Table I reveals that at a bath temperature of 45° C. the mean excess oxygen uptake observed during reactive hyperemia was much less than the mean predicted oxygen debt incurred in the previous period of arterial occlusion (an average of 0.804 cc. per 100 cc. limb volume, as compared with 2.021 cc.). That this difference was significant was substantiated by a statistical analysis of the data ($p < 0.001$).

At a bath temperature of 17° C., however, the mean excess oxygen was 0.410 cc. per 100 cc. of limb volume, as compared with a predicted value of 0.348 cc. A statistical analysis of these data revealed no significant difference between the two sets of figures ($p > 0.1$).

DISCUSSION

The present study supports the view, previously expressed (4), that an experimentally produced rise in tissue temperature of the forearm elicits an increase in resting oxygen uptake, while a fall results in a decrease in this figure (Table I). The results further demonstrate that this type of relationship also exists for the magnitude of the oxygen debt developed in a period of arterial occlusion and subsequently repaid during reactive hyperemia. Since the differences at the two bath temperatures were not associated with any consistent systemic reactions, it is probable that they were in great part due to alterations in local mechanisms brought about by the changes in tissue temperature.

At both tissue temperatures the oxygen debt incurred by a five minute period of arterial occlusion appeared to be repaid primarily through an increased blood flow, while the mechanism of greater extraction of oxygen from each cc. of blood passing through the tissues played a role only in the first minute or less of the period of reactive hyperemia. In fact, in a number of instances at both tissue temperatures, there was very little change in oxygen arteriovenous difference, in contrast with the large augmentation of blood flow that invariably occurred with re-establishment of the circulation. These findings are in accord with those reported on the dog (9).

The larger excess oxygen uptake during reactive hyperemia at a tissue temperature of 39.3° C., as compared with that at 26.8°, can be accounted for chiefly by more marked local circulatory changes occurring at the higher temperature. For example, under the latter circumstances, the height of the maximum peak of the blood flow curve in excess of the resting level was greater, the duration of the increase in blood flow was longer, and, most important, the excess quantity of blood entering the limb during reactive hyperemia was much larger than in the case of the lower tissue temperature.

There is a possibility, too, that at the higher tissue temperature the greater oxygen debt incurred in the period of arterial occlusion was satisfied in part by more effective removal of oxygen from each cc. of blood. In support of this view is the finding that in most of the experiments the initial increase in oxygen arteriovenous difference during reactive hyperemia was larger than at the low tissue temperature. Furthermore, there were fewer instances of a fall of the curve

below the baseline, indicating a smaller than resting oxygen arteriovenous difference.[5]

Although the present study leaves no doubt as to the penetrating effect of topically applied heat and cold, it is necessary to point out that under resting conditions all the tissues of the forearm were not equally affected by these agents. Actually there was a gradient of vasodilatation or constriction, with the greatest change occurring in the cutaneous vessels and the least, in the arterial tree located in the deepest muscle bundles.

During reactive hyperemia the situation was altered. At a bath temperature of 45° C. this state was associated primarily with further vasodilatation of arteries and arterioles in the deeper muscle layers, while the cutaneous vessels, already almost fully dilated by the heat, played little if any role in the response. The vascular contribution of the intervening tissues varied inversely with the distance from the surface of the limb.

The presence of such variations in vascular responses during reactive hyperemia constituted an objection to the use of blood from superficial tissues for the determination of representative figures of venous oxygen content, particularly at a high bath temperature. Under the latter circumstances the skin and subcutaneous tissue, having suffered from an oxygen debt during the previous period of arterial occlusion, would now remove large quantities of oxygen from the blood to satisfy this state. At the same time, however, the minimal vascular changes taking place in these tissues during reactive hyperemia would lead to a much greater depletion of oxygen from each cc. of blood than was occurring in the deep structures in which there was a simultaneous marked increase in circulation. It was because of this theoretical possibility that attempts were made to collect blood samples from veins which appeared to drain primarily deeper tissues. It was felt that oxygen arteriovenous differences thus obtained could with more basis be related to the blood flow changes taking place during reactive hyperemia, in order to arrive at an accurate figure for oxygen uptake.

It is of interest to speculate on the explanation for the significant difference observed at a bath temperature of 45° C. between the actual excess oxygen uptake during reactive hyperemia and the predicted oxygen debt for the preceding period of arterial occlusion, and the similarity of these two readings at a bath temperature of 17°. Several possibilities suggest themselves. First, at a bath temperature of 45° most of the components of the vascular tree are markedly dilated and, as a result, are filled with a considerable volume of blood, in part, also due to the large arterial inflow. Hence, when the arterial occlusion pressure is suddenly applied, a large complement of blood is trapped in the arterial tree, the capillaries and the venules, some of which is then available as a source of tissue oxygen during the period of arterial occlusion. In other words, limited aerobic metabolism is possible under these circumstances. As a result, a smaller than anticipated oxygen debt would be incurred; this, in turn, being reflected in a decreased oxygen repayment during reactive hyperemia. Such a hypothesis is in part supported by the observation of Patterson (10) who reported that when the forearm was packed with blood preceding the application of an arterial occlusion pressure, there was a reduction in the magnitude of the blood flow repayment in the subsequent period of reactive hyperemia.

At a bath temperature of 17° C. many of the superficial arterioles and capillaries are markedly constricted, and the total arterial inflow is low. As a consequence, the amount of blood that could be trapped in the forearm on the application of an arterial occlusion pressure, and thus considered as a potential source for aerobic metabolism, is small. Therefore, it would be expected that the magnitude of the oxygen debt developed in this period and subsequently repaid during reactive hyperemia would more closely approximate the predicted figure.

Another explanation that can be offered is that during a period of arterial occlusion the energy needs of the tissues are in part taken care of through anaerobic metabolism. On this basis, it is possible that at a bath temperature of 45° C., on re-establishment of the circulation some of the lactic acid, among other substances formed under such circumstances, is washed out of the forearm by the very rapid inflow of blood and resynthesized in the presence of oxygen in distant organs, as for

[5] It is possible that at both tissue temperatures this type of response represented a situation in which the increase in blood flow at that moment was of greater magnitude than warranted for the satisfaction of the existing metabolic needs of the tissues.

example, the liver. As a consequence, the local oxygen debt satisfied during reactive hyperemia would be smaller than the predicted figure by the amount repaid elsewhere.

At a bath temperature of 17° C., however, the situation is somewhat different. Because the initial blood flow response during reactive hyperemia is not as great as that at the higher bath temperature (average peak reading of 8.0 cc. per minute per 100 cc. limb volume, as compared with 18.4 cc.), the washing-out process with regard to products of anaerobic activity would be much less effective. As a result, the importance of distant synthesis would tend to be minimized, and, consequently, the actual oxygen uptake of the tissues of the forearm during reactive hyperemia would be expected to approach the predicted oxygen debt for the period of arterial occlusion.

Another possibility is that since the resting oxygen uptake was calculated on the basis of blood which drained primarily deep structures, the reported readings at a bath temperature of 45° C. were somewhat greater than a theoretical value which would have represented all the vascular tissues of the forearm (4). Under such circumstances, the predicted oxygen debt would also have to be considered larger than warranted. At the same time, the reading for oxygen repayment during reactive hyperemia was probably smaller than the true value because of the use of a resting oxygen uptake baseline in the derivation of this figure which was too high. However, it is doubtful whether such corrections, if they could have been made, would have materially altered the great difference observed between the predicted oxygen debt and the excess oxygen repayment.

Finally, certain technical difficulties may also have contributed to the findings. For example, if, instead of the 10 to 11 blood samples collected in the postocclusion period, it had been possible to obtain almost continuous measurement of venous oxygen content, the resulting curve of oxygen uptake would unquestionably have been depicted more exactly.[6] Perhaps, under such circumstances, the area under it would have been larger. On the other hand, at the low bath temperature, it is possible that the need for frequently repeated venous samples was not as great, in view of the less rapid and less marked responses during reactive hyperemia. On this basis, the observed curve of oxygen uptake could be considered to be a more accurate representation of the changes occurring in this period than in the case of the high bath temperature.

[6] Early in the investigation the use of an oximeter and a cuvette was considered as a possible means of obtaining frequent readings, but it was found that with this method, accurate determinations were not possible in the case of venous blood samples collected early in reactive hyperemia because of their low oxygen content.

SUMMARY AND CONCLUSIONS

1) The effect of altering local external temperature on the oxygen uptake of the tissues of the forearm during reactive hyperemia was investigated in a series of 27 experiments on 23 healthy subjects.

2) Oxygen uptake was calculated for the periods before and after five minutes of arterial occlusion, on the basis of studies of blood flow, using the venous occlusion plethysmographic method, and of local oxygen arteriovenous difference.

3) With prolonged exposure of the forearm to a bath temperature of 45° C., the mean tissue temperature rose from a control level of 36.0° C. (obtained with the limb in contact with air), to 39.3°. After exposure to a bath temperature of 17°, the readings fell to an average of 26.8°.

4) During the period of reactive hyperemia at an average tissue temperature of 39.3° C., the oxygen uptake in excess of the resting level was an average of 0.804 cc. per 100 cc. of limb volume, as compared with 0.410 cc. at a tissue temperature of 26.8°. This difference was found to be statistically significant.

5) At the high tissue temperature the average excess oxygen uptake during reactive hyperemia was found to be significantly less than the predicted oxygen debt for the previous period of arterial occlusion (0.804 cc. per 100 cc. limb volume, as compared with 2.021 cc.), while at the low tissue temperature both figures were approximately the same (0.410 cc. per 100 cc. limb volume, as compared with 0.348 cc.). An attempt was made to explain such findings.

6) Evidence was presented suggesting that at both bath temperatures the mechanism of repayment of an oxygen debt incurred in a period of arterial occlusion involved primarily an increase

in local circulation, with the factor of greater removal of oxygen from the blood playing a secondary role.

It was concluded that experimentally depressing the local tissue temperature of the forearm from an artificially elevated level definitely decreases the oxygen debt incurred in a period of arterial occlusion and subsequently repaid during reactive hyperemia. The data were considered to support and strengthen the view, previously expressed, that increasing local temperature of tissues *in vivo* raises their oxygen uptake, while decreasing it has the opposite effect.

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PULMONARY FUNCTION IN OBESE PERSONS[1,2]

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The purpose of this paper is to report a study of lung function in extremely obese persons. In 1936, Kerr and Lagen (2) called attention to the fact that some obese persons develop dyspnea, cyanosis, polycythemia, and cardiac failure. They thought that these signs and symptoms developed because of mechanical interference with ventilation, especially movement of the diaphragm. Sieker, Estes, Kelser and McIntosh (3) reported studies in four obese patients and postulated a cardiorespiratory syndrome peculiar to obese persons and characterized by somnolence, Cheyne-Stokes respirations, intermittent cyanosis, and polycythemia. At the same time that Sieker and his associates were working on this problem, Auchincloss, Cook and Renzetti (4) and Weil and Prasad (5) observed a peculiar type of polycythemia associated with obesity. Most of the investigators previously mentioned have reported additional patients characterized by obesity, hypoxemia, polycythemia and alveolar hypoventilation (6–9), and they have been joined by others (10–18). Burwell, Robin, Whaley and Bickelmann (12) have given the picturesque name "Pickwickian Syndrome" to this combination of signs and symptoms.

There is agreement that the cardinal characteristics of these patients are obesity, alveolar hypoventilation, and polycythemia. The basic physiologic defect is alveolar hypoventilation. This leads to arterial hypoxemia and hypercapnia. Arterial hypoxemia provokes polycythemia. Therefore, the polycythemia of these patients is no longer of a mysterious type but is secondary to hypoxemia.

No agreement exists on what initiates alveolar hypoventilation. Several theories have been advanced. One is that the amount of work necessary to keep the arterial carbon dioxide pressure (P_{CO_2}) at a normal level is too great. A compromise is made which allows the P_{CO_2} to rise rather than to employ the continuous excess effort necessary to keep the P_{CO_2} normal (13, 15). Another is that in some persons there is a critical degree of obesity at which ventilatory insufficiency appears (12). Still another is that massive obesity restricts pulmonary ventilation and leads to alveolar hypoventilation (5, 8, 9).

We decided to study a number of obese persons to determine the frequency of various abnormalities and to investigate the mechanism of alveolar hypoventilation. We are reporting studies on 28 obese persons with restudy of 16 following weight loss.

METHODS

The patients were selected from the wards and clinics of the University Hospitals and Veterans Administration Hospital, Iowa City. We required that all patients be at least 100 pounds over ideal weight based on height and size of subject's frame (19). Each patient had a complete history, physical examination, roentgenogram of the chest, and the indicated laboratory tests. Pulmonary function tests were done in the morning after the patient had eaten breakfast. The vital capacity, inspiratory capacity and expiratory reserve volume were measured separately using a Benedict Roth spirometer. The maximum of at least three trials was recorded. The functional residual capacity of the lungs was measured in duplicate by the nitrogen washout method of Darling, Cournand and Richards (20). Volumes were corrected to body temperature, ambient pressure, and saturated with water vapor, hereafter called B. T. P. S. Predicted normal values for lung volumes were calculated on the basis of the patient's height (21) rather than the body surface area. Rate, depth and minute volume of ventilation were measured while the patient was breathing air, 99.6 per cent oxygen, and 7.5 per cent carbon dioxide in air. Expired volumes were collected in a Douglas bag or a Tissot spirometer simultaneously with the drawing of samples of arterial blood. Volumes were corrected to B. T. P. S. Physiologic dead space was calculated using Bohr's equation. Measured and derived values used

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[2] Supported by a grant from the Iowa Tuberculosis and Health Association.

in this calculation are the volume of expired air over a three minute period, the per cent CO_2 in the expired air, and the arterial P_{CO_2}. Calculation is based on the formula given by Comroe, Forster, DuBois, Briscoe and Carlsen (22). Uniformity of intrapulmonary distribution of inspired gas was checked by the single breath nitrogen test of Comroe and Fowler (23) and the measurement of the percentage of nitrogen at the end of seven minutes breathing of oxygen as described by Cournand, Baldwin, Darling and Richards (24). To measure maximal breathing capacity (MBC), the subject breathed for 15 seconds through a mouthpiece, low resistance valve, and wide tubing into a Tissot spirometer. Predicted normal values for the MBC were based on age (25). The highest value obtained with three trials was calculated in liters per minute. Maximal inspiratory (or expiratory) flow rate was measured between 200 and 1,200 ml. of the inspiration (or expiration) according to the method of Danzig and Comroe (26). Diffusion was studied using the single breath carbon monoxide method of Ogilvie, Forster, Blakemore and Morton (27).

Arterial blood was obtained from the brachial artery while the semirecumbent subject breathed air, and again after he had breathed 99.6 per cent oxygen for at least 10 minutes. The samples were analyzed for total oxygen content, oxygen capacity, and whole blood carbon dioxide content by the manometric technique of Van Slyke and Neill (28). Blood was rotated with air in a tonometer at room temperature for 20 minutes before measuring oxygen capacity. Appropriate corrections were made for physically dissolved oxygen and for differences in total hemoglobin in the content and capacity samples. Direct measurement of dissolved oxygen was not made, but an approximate calculation was made on the samples collected during the inhalation of oxygen by subtracting the oxygen capacity from the total oxygen content of such blood. Arterial blood pH was measured in a closed Cambridge glass electrode at either 37° C. or, if done at room temperature, the pH value was corrected to 37° C. using the factor of Rosenthal (29). Plasma carbon dioxide content and P_{CO_2} were determined from pH, hematocrit, oxygen saturation and whole blood carbon dioxide content using the nomogram of Singer and Hastings (30).

Some of our patients had edema of the legs thought to be caused by congestive heart failure. When these patients decreased their activity after hospitalization and had appropriate therapy for heart failure, they promptly lost from 5 to 30 pounds which probably represented water loss. Our "initial" studies were done after this rapid loss of water in most cases. When the initial studies were completed, we placed the patients on an 800 calorie diet and later restudied those who lost a significant amount of weight. Digitalis, salt restriction, and diuretics were used only when indicated. Weight reduction was best accomplished by continuous hospitalization of these patients. When possible, patients were hospitalized until they had lost 50 to 100 pounds. In some cases this took six months.

RESULTS

The patients are divided into two groups, depending on arterial oxygen saturation at rest. Arterial hypoxemia is considered to be present when arterial saturation is below 93.2 per cent (31). Thirteen patients had normal saturation and are considered in Group I. Fifteen patients had arterial hypoxemia and constitute Group II.

A. Patients with normal arterial oxygen saturation

This group consists of 13 patients whose weight averaged 303 pounds at the time of initial study. There were 6 men and 7 women. The age range was 19 to 67 years. The results of pulmonary function studies in these patients are listed in Tables I and II. Lung volumes were normal. Mean inspiratory capacity was 92 per cent of predicted normal; mean expiratory reserve volume was 75 per cent of predicted normal; mean vital capacity was 89 per cent of predicted normal; mean residual volume was 136 per cent of predicted normal; and mean total lung capacity was 100 per cent of predicted normal. Mean minute volume of ventilation was 9.9 liters and mean minute volume of alveolar ventilation was 6.2 liters. The physiologic dead space averaged 218 ml. Distribution of inspired air was slightly uneven as measured by the single breath nitrogen test, but normal as measured by the less sensitive test based on the per cent of nitrogen at the end of seven minutes of oxygen breathing. Mechanical tests showed mild abnormalities. The maximal breathing capacity was slightly below normal, the mean value being 87 per cent of predicted normal. The maximal expiratory flow rate averaged 212 liters per minute while the maximal inspiratory flow rate averaged 156 liters per minute. Diffusion studies done in nine patients were normal.

Six patients were restudied after weight loss. These six patients averaged 304 pounds at the time of original study and 252 pounds at the time of the last study. Weight loss changed the lung volumes only slightly with the exception of the expiratory reserve volume which increased from a mean value of 63 per cent of predicted normal to a mean value of 102 per cent of predicted normal. Minute volume of ventilation was essentially unchanged: 10.0 liters before weight loss and 10.2 liters after weight loss. Mean alveolar ventilation

TABLE I

Physical characteristics and lung volumes in obese patients with normal arterial oxygen saturation

Patient	Age	Sex	Height (*inches*)	Weight (*pounds*)	Date of study	Lung volumes: Inspiratory capacity *ml.*	Inspiratory capacity %*	Expiratory reserve volume *ml.*	Expiratory reserve volume %*	Vital capacity *ml.*	Vital capacity %*	Residual volume *ml.*	Residual volume %*	Total lung capacity *ml.*	Total lung capacity %*	Residual volume/total lung capacity ratio ×100
D. C.	19	F	71	321	15 Nov. 56	3,100	80	1,070	82	3,790	84	1,630	145	5,420	96	30
				274†	22 Jan. 57	2,790	76	1,470	113	4,370	103	1,510	134	5,770	103	26
R. W.	21	M	68	250	11 Dec. 56	3,500	108	1,590	147	5,110	119	1,750	162	6,860	127	26
D. V.	35	F	70	313	21 May 57	3,400	128	750	84	4,040	114	1,600	147	5,640	124	28
F. S.	38	M	72	314	6 Dec. 56	2,960	86	810	70	3,870	85	1,700	121	5,570	93	30
				270†	8 Feb. 57	2,580	75	1,400	122	4,030	88	2,350	206	6,380	105	37
F. P.	38	M	66	274	18 June 57	4,050	128	700	66	4,750	112	2,160	204	6,910	131	31
W. B.	39	M	70	382	26 July 56	2,960	89	570	52	3,440	78	1,170	123	5,110	89	33
M. F.	41	F	64	348	11 June 57	2,240	91	370	46	2,890	88	2,100	210	4,990	117	45
E. T.	47	F	69	305	23 May 57	2,480	94	1,370	157	3,820	109	2,130	199	5,950	131	36
F. H.	48	F	62	243	6 Dec. 56	1,880	63	480	51	2,790	71	1,040	86	3,830	74	27
N. S.	54	F	65	319	31 July 56	1,850	75	340	40	2,290	69	1,890	78	4,180	71	46
				260†	30 Apr. 57	1,920	78	940	114	2,470	75	2,260	93	4,730	83	48
J. R.	55	F	66	345	3 Sept. 57	2,200	88	480	57	2,610	78	1,290	53	3,900	68	33
				306†	5 Nov. 57	2,460	98	700	83	2,890	87	1,080	44	3,970	69	27
R. G.	61	M	68	254	3 Dec. 56	2,730	84	600	55	3,190	73	2,430	100	5,620	83	43
				175†	16 May 57	2,740	84	890	82	3,600	83	1,980	81	5,580	83	35
R. M.	67	M	68	272	9 Aug. 56	2,680	83	770	72	3,390	79					
				229†	21 Mar. 57	2,160	67	1,080	100	3,700	86	3,570	147	7,270	108	52
Mean, (initial) 13 patients				303			92		75		89		136		100	
Mean, (before weight loss) 6 patients				304			83		63		78		99‡		82‡	
Mean, (after weight loss) 6 patients				252			80		102		87		112‡		89‡	

* Per cent of predicted value based on height (21).
† Indicates weight after weight loss at the time of repeat study.
‡ Mean value represents only five patients.

TABLE II

Studies of ventilation, respiratory mechanics, and diffusion in obese patients with normal arterial oxygen saturation

Patient	Ventilation					Mechanical tests				
	Minute volume			Alveolar gas distribution		Maximal breathing capacity		Maximal expiratory flow rate	Maximal inspiratory flow rate	Diffusion
	Total *L.*	Alveolar *L.*	Physiologic dead space *ml.* % *	7 min. washout (% N_2)†	Single breath N_2 test (% N_2)‡	*L./min.*	%§	*L./min.*	*L./min.*	*ml. CO/mm. Hg/min.*
D. C.	5.9			0	1.7	100	79	171	171	
	4.5	2.4	122 (33)	1.7	2.0	117	93	158	158	31
R. W.	8.3	5.4	249 (42)	0	1.5	115	91	250	90	
D. V.	13.5	9.0	265 (33)	2.0	1.5	115	129	250	188	32
F. S.	7.9	6.2	145 (29)	0	2.3	68	62	214	300	
	14.2	9.2	469 (43)	1.0	2.3	84	77	222	300	28
F. P.	10.5	6.7	238 (36)	1.8	1.0	114	105	300	171	37
W. B.	11.0	7.2	204 (41)	1.4	0	89	81	250	151	
M. F.	10.7	6.0	170 (36)	1.5	1.0	63	71	167	146	
E. T.	10.6	7.3	254 (31)	2.2	1.7	87	98	176	167	40
F. H.	3.4	2.1	105 (44)	0	2.0	69	77	207	81	
N. S.	13.4	7.3	282 (46)	4.1	2.0	63	85	103	101	
	10.5	8.2	135 (22)	2.0	2.0	70	95	92	92	20
J. R.	9.6	5.3	250 (51)	0.2	2.1	70	96	250	176	27
	9.0	4.1	258 (55)	0.7		86	118	260	174	24
R. G.	10.4	5.6	199 (54)	0	7.5	74	81	200	98	
	14.3	6.8	270 (53)	5.0	2.7	108	119	300	230	14
R. M.	12.6	6.9	253 (45)	0.6	1.9	64	71	214	179	
	8.7	5.5	188 (47)	0.3	2.0	64	71	222	188	43
Mean, (initial) 13 patients	9.9	6.2	218 (41)	1.0	2.0		87	212	156	
Mean, (before weight loss) 6 patients	10.0	6.3‖	226 (45)‖	0.8	3.1‖		79	192	171	
Mean, (after weight loss) 6 patients	10.2	6.8‖	264 (44)‖	1.8	2.2‖		96	209	190	

* Per cent of tidal volume.
† Normal values for seven minute nitrogen washout are less than 2.5 per cent N_2.
‡ Normal values for single breath nitrogen test are less than 1.5 per cent N_2.
§ Per cent of predicted value based on age (25).
‖ Mean value represents only five patients.

increased from 6.3 liters to 6.8 liters in five patients. Alveolar gas distribution was practically unchanged (the per cent nitrogen after seven minutes of oxygen breathing averaged 0.8 per cent before weight loss and 1.8 per cent after; the single breath nitrogen test averaged 3.1 per cent before weight loss and 2.2 per cent after weight loss). Weight loss increased the mean maximal breathing capacity from 79 per cent of predicted normal to 96 per cent of predicted normal. Maximal flow rates were also increased; the mean expiratory rate increased from 192 liters per minute to 209 liters per minute and the mean inspiratory rate increased from 171 liters per minute to 190 liters per minute.

The results of arterial blood studies in these patients are listed in Table III. Mean arterial oxygen saturation at rest was 96.0 per cent, mean P_{CO_2} was 39 mm. Hg, mean pH was 7.40, and the mean hematocrit was 46 per cent. Weight loss in six patients made no change except that the hematocrit decreased from a mean value of 46 per cent to 41 per cent.

B. Patients with arterial hypoxemia

This group is composed of 15 patients whose mean weight was 315 pounds and who had arterial hypoxemia. There were 12 women and 3 men. Their ages ranged from 39 to 67 years. Ten of the 15 patients had clinical or laboratory evidence of lung disease. Two women had bronchial asthma; two men had pulmonary emphysema; one man had multiple pulmonary infarcts; two women had pulmonary edema secondary to heart disease; three women had no clinical evidence of lung disease but laboratory evidence suggested it. Two women had myxedema, and three women had hypoxemia without evidence of lung disease. The initials, clinical diagnoses, physical characteristics, and results of pulmonary function tests in these patients are listed in Tables IV and V. The results of arterial blood studies are listed in Table VI. Ten patients were restudied after weight reduction. The average initial weight in these 10 patients was 323 pounds and at the time of last study it was 251 pounds, a mean loss of 72 pounds.

TABLE III

Arterial blood studies in obese patients with normal arterial oxygen saturation

Patient	Date of study	O_2 content (rest) *vol. %*	O_2 capacity *vol. %*	O_2 saturation (Rest) %	O_2 saturation 100% O_2* %	P_{CO_2} (rest) *mm. Hg*	pH (rest)	Hematocrit %
D. C.	15 Nov. 56	15.30	15.43	99.2	100 + 2.27	34	7.44	39
	22 Jan. 57	13.69	14.25	96.1	100 + 1.91	39	7.39	34
R. W.	11 Dec. 56	18.14	18.82	96.4	100 + 2.11	33	7.46	53
D. V.	21 May 57	15.95	16.47	96.8	100 + 2.03	42	7.35	41
F. S.	6 Dec. 56	19.70	20.89	94.3	100 + 1.35	36	7.46	51
	8 Feb. 57	17.99	17.66	100 + 0.33	100 + 2.70	35	7.41	45
F. P.	18 June 57	19.56	20.52	95.3	100 + 0.87	38	7.39	48
W. B.	26 July 56	18.66	19.07	97.9	100 + 2.56	42	7.39	52
M. F.	11 June 57	18.71	19.39	96.5	100 + 1.56	40	7.34	47
E. T.	23 May 57	15.51	16.18	95.9	100 + 1.83	46	7.34	39
F. H.	6 Dec. 56	17.71	18.92	93.6	100 + 1.20	38	7.44	56
N. S.	31 July 56	20.61	23.03	93.6		33	7.41	52
	30 Apr. 57	16.93	17.60	96.2	100 + 1.78	31	7.48	43
J. R.	3 Sept. 57	16.82	17.95	93.7	100 + 1.54	46	7.36	42
	5 Nov. 57	17.11	18.41	93.0	100 + 1.73	46	7.35	42
R. G.	3 Dec. 56	18.46	18.71	98.7	100 + 2.34	35	7.41	50
	16 May 57	15.05	16.14	93.3	100 + 1.62	37	7.40	41
R. M.	9 Aug. 56	16.78	17.48	96.0	100 + 2.11	37	7.35	40
	21 Mar. 57	17.00	17.41	97.7	100 + 1.46	37	7.39	43
Mean, (initial) 13 patients				96.0		39	7.40	46
Mean, (before weight loss) 6 patients				95.9		37	7.41	46
Mean, (after weight loss) 6 patients				96.1		38	7.40	41

* Values following + sign refer to ml. O_2 per 100 ml. blood in excess of that required to saturate hemoglobin (*i.e.*, dissolved O_2). Normal value for dissolved O_2 is 2.00 ml.

TABLE IV

Physical characteristics and lung volumes in obese persons with hypoxemia

Patient	Clinical diagnosis	Age	Sex	Height (*inches*)	Weight (*pounds*)	Date of study	Lung volumes										Residual volume/ total lung capacity ratio ×100
							Inspiratory capacity		Expiratory reserve volume		Vital capacity		Residual volume		Total lung capacity		
							ml.	%*	*ml.*	%*	*ml.*	%*	*ml.*	%*	*ml.*	%*	
A. H.	Bronchial asthma	39	F	64	317	14 June 56	1,660	68	390	48	1,950	60	2,390	240	4,340	102	55
					208	21 Feb. 57	1,345	55	510	63	1,880	58	1,650	166	3,530	82	47
I. K.	Bronchial asthma	51	F	61	349	18 Sept. 56	1,030	44	670	87	1,590	51	2,020	83	3,610	65	56
					285	7 Jan. 57	1,110	48	950	122	1,890	61	2,210	91	4,100	74	54
					254	18 Mar. 57	1,300	56	1,130	146	2,170	70	2,610	107	4,780	86	55
					233	28 May 57	1,520	65	1,230	159	2,310	74	2,280	94	4,590	83	50
					200	11 Nov. 57	1,750	75	1,240	160	2,710	87	1,890	78	4,600	83	41
W. C.	Pulmonary emphysema	48	M	69	237	21 Sept. 56	1,725	53	740	66	2,350	54	2,800	210	5,150	90	53
					211	25 Oct. 56	2,120	65	870	77	2,730	62	2,750	206	5,480	95	48
W. B.	Pulmonary emphysema	44	M	71	289	26 Nov. 56	2,650	79	1,190	106	3,190	71	3,270	247	6,460	110	51
					232	23 Apr. 57	2,545	75	1,180	104	3,720	83	3,480	262	7,200	122	48
H. B.	Pulmonary infarcts	45	M	65	268	13 Dec. 56	840	27	130	13	980	24	2,750	218	3,730	69	74
F. W.	Aortic stenosis—pulmonary edema	62	F	62	243	24 June 57	1,670	71	510	65	2,130	67	2,450	101	4,630	83	53
I. F. K.	Arteriosclerotic heart disease—pulmonary edema	52	F	64	322	24 Jan. 57	1,730	71	220	27	1,920	59	1,510	62	3,430	60	44
					250	6 Aug. 57	2,340	99	430	55	2,890	91	2,280	94	5,170	92	44
L. R.	Myxedema	67	F	69	388	14 Mar. 57	1,240	47	410	45	1,550	44	1,390	57	2,940	50	50
					245	21 Nov. 57	1,760	72	1,360	167	2,990	92	940	39	3,930	69	24
K. P.	Myxedema	41	F	65	318	3 Sept. 57	2,340	94	430	52	2,750	83	910	36	3,660	62	25
E. B.	Essential hypertension—pulmonary disease, type not determined	54	F	64	366	8 Dec. 55	1,330	55	440	54	1,750	54	2,060	85	3,810	67	54
					335	19 Apr. 56	1,720	70	530	65	1,730	53	2,010	83	3,740	66	54
A. L.	Pulmonary disease, type not determined	39	F	64	280	16 Feb. 56	1,330	55	270	33	1,460	45	1,850	187	3,310	78	56
					234	6 Mar. 56	1,550	64	590	73	2,090	65	2,910	294	5,000	119	57
O. R.	Pulmonary disease, type not determined	42	F	62	342	15 Aug. 57	1,060	45	740	94	1,456	46	1,980	204	3,430	83	58
					300	15 Oct. 57	2,025	85	810	102	2,610	82	2,010	207	4,620	117	41
H. H.	No lung disease	51	F	65	337	7 Mar. 57	2,075	80	550	66	2,570	78	2,100	86	4,670	82	44
					296	15 July 57	1,995	77	1,270	148	3,280	95	1,850	76	5,130	86	36
A. D.	No lung disease	52	F	65	414	27 Aug. 57	2,870	116	270	33	3,120	95	1,390	57	4,530	77	34
M. F.	No lung disease	62	F	67	255	27 June 57	2,860	87	300	27	3,260	73	2,190	90	5,340	78	41
Mean (initial) 15 patients					315												
Mean (before weight loss) 10 patients					323												
Mean (after weight loss) 10 patients					251												

* Per cent of predicted value based on height (21).

TABLE V

Studies of ventilation, respiratory mechanics, and diffusion in obese patients with hypoxemia

	Ventilation					Mechanical tests				
	Minute volume			Alveolar gas distribution		Maximal breathing capacity		Maximal expiratory flow rate	Maximal inspiratory flow rate	Diffusion
Patient	Total *L.*	Alveolar *L.*	Physiologic dead space *ml.* (%)*	7 min. washout % N_2†	Single breath N_2 test % N_2‡	*L./min.*	%§	*L./min.*	*L./min.*	*ml. CO/ mm. Hg/ min.*
A. H.	10.2	7.5	161 (32)	3.8	7.0	41	46	55	82	
	6.8	3.5	143 (49)	2.3	1.5	41	46	63	109	
I. K.	9.3	3.6	242 (68)	5.0	1.8	33	45	34	63	
	7.5	3.2	204 (69)	4.0	2.5	42	57	26	146	25
	6.8	3.3	135 (52)	1.5	2.8	37	50	22	162	27
	10.3	5.9	165 (44)	2.2	2.4	42	57	45	182	
	8.4	4.1	183 (44)	1.8	4.5	45	61	53	154	26
W. C.	7.4	4.0	153 (52)	13.2	6.5	32	36	34	111	
	7.6	4.1	209 (55)	2.1	5.3	46	51	97	140	
W. B.	7.6	3.3	272 (65)	0.7	8.5	51	47	72	109	
	11.7	5.9	363 (50)	0.3	3.3	66	61	105	143	18
H. B.	6.4	1.9	150 (78)			25	23	35	83	
F. W.	7.3	4.4	223 (40)	2.5	1.5	75	101	122	167	12
I. F. K.	6.2	5.0	97 (31)	1.9	0.5	48	65	158	75	12
	8.0	5.2	153 (35)	1.8	0.9	84	113	214	188	35
L. R.	8.5	2.6	126 (70)	1.3	1.3	70	95	46	64	10
	10.6	4.4	295 (59)	0.4	0.8	111	135	286	125	15
K. P.	7.7	3.7	200 (42)	1.7	2.2	57	64	207	136	20
E. B.	10.0			1.8	2.5	57	78	47	88	
	9.0	4.1	221 (54)	1.5	6.0	40	54	43	61	
A. L.	7.0	3.2	165 (59)	7.0		27	30	31	86	
	9.0	6.3	121 (30)	6.3	6.3	43	37	59	128	
O. R.	7.3	4.0	145 (40)	1.4	6.5	40	45	110	154	15
	9.0	6.0	300 (33)	0.2	3.9	79	89	188	115	16
H. H.	7.1	3.9	128 (45)	0.2	0.5	45	61	250	188	34
	10.6	5.4	208 (49)	0.4	1.6	97	131	285	220	27
A. D.	10.5	4.8	329 (54)	0.4	1.5	83	113	231	160	20
M. F.	8.0	5.0	214 (48)	1.0	0.9	105	142	250	97	19

* Per cent of vital capacity.
† Normal values for seven minute nitrogen washout are less than 2.5 per cent N_2.
‡ Normal values for single breath nitrogen test are less than 1.5 per cent N_2.
§ Per cent of predicted value based on age (25).

The asthmatic patients had reduced vital capacities, normal or increased residual volumes, uneven distribution of inspired air, diminished maximal breathing capacities and maximal flow rates, arterial hypoxemia, and carbon dioxide retention. Following weight loss of 109 pounds, Patient A. H. was unimproved as measured by pulmonary function studies and arterial blood studies. Patient I. K. lost 149 pounds. She had the following evidence of improvement: Vital capacity changed from 51 per cent to 87 per cent of predicted normal, arterial oxygen saturation increased from 90 per cent to 98 per cent and P_{CO_2} changed from 66 to 44 mm. Hg. This patient has been studied five times over a period of 14 months; the studies demonstrate gradual progressive improvement with continued weight loss (see Tables IV, V, and VI).

The two patients with pulmonary emphysema had reduced vital capacities, increased residual volumes, uneven distribution of inspired air, reduced maximal breathing capacities, and maximal expiratory flow rates, hypoxemia, and carbon dioxide retention. Following weight loss of 26 pounds, Patient W. C. had improvement in arterial oxygen saturation, decrease in carbon diox-

ide retention, more even distribution of inspired air, and improvement in the measurements of mechanical function. This man was probably benefited in part also by phlebotomy, salt restriction, and mercurial diuretics. Patient W. B. lost 57 pounds in weight and improved similarly. His arterial oxygen saturation and P_{CO_2} returned to normal.

The patient with multiple pulmonary infarcts had severe hypoxemia and carbon dioxide retention. On breathing 99.6 per cent oxygen for 10 minutes, his arterial blood did not become fully saturated indicating that a sizable amount of blood was not coming into contact with ventilated alveoli before entering the left heart. His vital capacity was 24 per cent of predicted normal, his alveolar ventilation was 1.9 liters per minute, and the results of tests of mechanical function were abnormally low. The diagnosis of multiple pulmonary infarcts was proved at necropsy.

Patients I. F. K. and F. W. who had pulmonary edema and L. R. and K. P. who had myxedema had similar results. They had reduced vital capacities, normal or reduced residual volumes, and reduced total lung capacties. All four had normal distribution of inspired air but low diffusing capacities. Tests of mechanical function were normal or only slightly impaired. All four patients had arterial hypoxemia and three patients had carbon dioxide retention. Following weight loss of 72 pounds, I. F. K. had normal arterial oxygen saturation, increased vital capacity

TABLE VI

Arterial blood studies in obese patients with hypoxemia

Patient	Date of study	O_2 content (rest) *vol. %*	O_2 capacity *vol. %*	O_2 saturation (Rest) %	O_2 saturation 100% O_2* %	P_{CO_2} (rest) *mm. Hg*	pH (rest)	Hematocrit %
A. H.	14 June 56	14.39	17.96	80.1	100 + 0.45	52	7.36	49
	21 Feb. 57	15.21	21.38	71.1	96.5	59	7.30	62
I. K.	18 Sept. 56	16.65	18.45	90.2	100 + 1.22	66	7.26	58
	7 Jan. 57	18.44	19.98	92.3	100 + 1.56	50	7.37	48
	18 Mar. 57	17.80	18.44	96.5	100 + 1.81	48	7.35	46
	28 May 57	17.30	17.27	100 + 0.03	100 + 2.15	39	7.42	43
	12 Nov. 57	15.97	16.25	98.3	100 + 2.33	44	7.38	41
W. C.	21 Sept. 56	20.29	24.86	81.6	100 + 2.56	63	7.31	72
	25 Oct. 56	19.16	20.21	94.8	100 + 1.77	50	7.34	52
W. B.	28 Nov. 56	20.16	23.41	79.3	100 + 0.06	51	7.38	65
	23 Apr. 57	16.57	16.66	99.5	100 + 2.50	42	7.37	45
H. B.	13 Dec. 56	6.68	17.88	37.4	89.4	98	6.99	50
F. W.	24 June 57	14.85	17.44	85.2	100 + 0.91	47	7.38	47
I. F. K.	24 Jan. 57	17.56	19.54	89.9	100 + 1.77	32	7.53	50
	6 Aug. 57	16.48	17.40	94.7	100 + 1.10	40	7.39	40
L. R.	14 Mar. 57	12.34	15.01	82.2	100 + 1.70	54	7.31	41
	21 Nov. 57	13.96	14.64	95.4		42	7.36	37
K. P.	3 Sept. 57	12.99	14.33	90.6	100 + 0.75	49	7.34	37
E. B.	8 Dec. 55							
	19 Apr. 56	12.55	13.98	89.8	100 + 1.32	45	7.37	36
A. L.	16 Feb. 56	9.25	17.78	52.0	100 + 1.49	70	7.37	53
	6 Mar. 56	18.23	22.08	87.3		46	7.39	60
O. R.	15 Aug. 57	13.97	18.30	76.3	100 + 1.60	48	7.36	50
	15 Oct. 57	16.27	18.83	86.4	100 + 1.00	48	7.36	51
H. H.	7 Mar. 57	14.99	16.54	90.6	100 + 0.71	45	7.36	41
	15 July 57	15.45	15.46	99.9	100 + 1.90	44	7.36	39
A. D.	27 Aug. 57	15.56	16.80	91.4	100 + 0.96	45	7.39	40
M. F.	27 June 57	17.19	18.60	92.4	100 + 1.49	40	7.38	43

* Values following + sign refer to ml. O_2 per 100 ml. blood in excess of that required to saturate hemoglobin (*i.e.*, dissolved O_2). Normal value for dissolved O_2 is 2.00 ml.

and residual volume, and improvement of maximal breathing capacity and maximal flow rates. Diffusion returned to normal. Patient L. R. lost 143 pounds and in addition received dessicated thyroid for myxedema. Repeat studies demonstrated normal arterial oxygen saturation, normal P_{CO_2}, only minor changes in lung volumes, increased alveolar ventilation, and improvement in the maximal breathing capacity and maximal flow rates. Diffusion returned toward normal. Patients F. W. and K. P. have not been restudied.

Three patients (E. B., A. L. and O. R.) had laboratory evidence of lung disease of unclassified type resembling pulmonary emphysema. They had reduced vital capacities, normal or increased residual volumes, uneven distribution of inspired air, reduced maximal breathing capacities, and severely reduced maximal flow rates with the expiratory flow rates being reduced out of proportion to the reduction in inspiratory flow rates. Diffusion was low in the one patient in whom it was measured. Study of the patients' arterial blood revealed hypoxemia and carbon dioxide retention. All three patients have been restudied after weight loss. Weight loss varied from 31 pounds in Patient E. B. to 46 pounds in Patient A. L. In two patients the vital capacity increased. The residual volume was unchanged in two patients but increased in one patient. Alveolar ventilation increased in all three patients. All three patients still have evidence of uneven distribution of inspired air. After weight loss, two patients were improved as measured by the maximal breathing capacity test; the other patient did worse on retesting. Maximal flow rates continue to be abnormal in all three patients. The diffusing capacity has not changed in the one patient in whom this was studied. Study of the arterial blood in two patients before and after weight loss demonstrated improved oxygen saturation in both, and a fall in P_{CO_2} toward normal in one.

Three patients (H. H., A. D. and M. F.) had arterial hypoxemia without evidence of lung disease. Patient H. H. lost 41 pounds in weight. This was associated with a return of arterial oxygen saturation to normal.

DISCUSSION

We sought to determine the frequency and nature of alveolar hypoventilation in obese persons. In 28 we found 10 who had alveolar hypoventilation as manifested by increased arterial P_{CO_2}. Five other obese persons had hypoxemia not associated with alveolar hypoventilation. Alveolar hypoventilation was associated with myxedema or lung abnormalities including bronchial asthma, emphysema, infarction, edema, and lung disease of unclassified type. Others (*32*, 33) have reported alveolar hypoventilation in obese patients who had normal lungs but who were thought to have a lesion of the medullary respiratory center. Disorders of the muscles of respiration such as poliomyelitis could produce an abnormal ventilatory response in obese persons and be associated with alveolar hypoventilation. We have no such cases but no doubt they exist.

The existence of alveolar hypoventilation in any patient must be caused by lung disease, malfunction of the chest bellows, inadequate neuro-muscular coordination, or a central nervous system lesion. The physiologic problem in the obese patient with hypoventilation is to identify in which of these four areas lies the derangement of function. Others have advanced the following theory to explain alveolar hypoventilation in obese people who have normal lungs. In the obese individual the work of breathing is increased (16, 34). Some increased work of breathing can be tolerated, but when obesity reaches a certain point the work of breathing becomes excessive and the patient adjusts to suboptimal ventilation by development of a refractory medullary state, thereby permitting alveolar hypoventilation. When weight is reduced, the work of breathing is reduced, normal medullary activity reappears, and the alveoli are properly ventilated. No one has demonstrated the point at which the work of breathing becomes so excessive that alveolar hypoventilation is preferable to a further increase in the work of breathing. Our group of obese patients with normal lungs consists of people between the ages of 19 and 67 years and weighing as much as 382 pounds. None of these persons had alveolar hypoventilation. Our obese persons with normal lungs tolerated the increased work load satisfactorily. We believe that the obese patient with normal lungs and a normal respiratory center may be able to move his chest enough to provide adequate ventilation. However, we still must deal with the mechanism of production of alveolar hypoventila-

tion in obese patients without disease of the lungs. Is it possible that obesity alone, by putting a severe load on the respiratory bellows, leads to a derangement of the respiratory center? Whether obesity alone is enough to produce such a lesion we cannot say. We have no evidence to support the theory that it does. Nor can we say categorically that it doesn't. If such patients exist we have not studied one, although we have searched diligently for one.

We suspect that the increased work of breathing when associated with even mild lung disease, malfunction of the muscles of respiration, or a central nervous system lesion is enough to produce alveolar hypoventilation and the events which follow. Others have shown that weight loss reduces the work of breathing (16) and improves alveolar ventilation. We believe that this mechanism is responsible for the improvement in lung function in our obese patients who lost weight. However, weight reduction alone does not correct underlying lung disease. One of our asthmatic patients (A. H.) lost weight but discontinued her cortisone. At this point pulmonary function tests showed that she was worse than before weight reduction.

Three of our patients had laboratory evidence of lung disease of unclassified type. This lung disease was characterized by reduced vital capacity, normal or increased residual volume, uneven distribution of inspired air, reduced maximal breathing capacity, reduced maximal flow rates with the expiratory flow rate being reduced out of proportion to the reduction in the inspiratory flow rate, and diminished diffusing capacity. Following weight loss, all three patients continued to have uneven distribution of inspired air and abnormal mechanical tests. These persistent abnormalities are consistent with a structural defect similar to that seen in local and diffuse pulmonary emphysema, although clinical evidence of either condition was not present. In these patients the pulmonary abnormalities were mild in degree but they were associated with hypoxemia and carbon dioxide retention. Pulmonary insufficiency could be reversed by weight reduction but evidence of lung disease persisted.

A possibility exists that the obese patient may have a lesion in one of the four areas previously mentioned which is transient, which results in temporary hypoventilation, and which eventually clears up regardless of whether or not the patient loses weight. This mechanism, a transient lesion in one of these four areas, for example a pulmonary infarct or edema of the medullary respiratory center, could explain some of the cases reported by others. Then, too, some of the patients reported by others were studied incompletely and thus the possibility of lung disease was not excluded. Other patients had "complete" studies, but this implies only that all available tests were done. Lung disease can exist in the presence of normal "complete" pulmonary studies.

Necropsies have been done on several patients. One patient had mild emphysema (17), one patient had bronchopneumonia (13), one patient had extensive thrombi in the pulmonary trunk and pulmonary arteries with infarction of the lower lobe of the right lung (15), and one patient had pulmonary artery atherosclerosis, focal areas of atelectasis, and recent pulmonary infarction (35). The patient studied by us (H. B.) who died had extensive pulmonary infarction.

Two of our patients with alveolar hypoventilation had myxedema. These patients may have had pulmonary edema to explain reduced vital capacity, reduced diffusing capacity, and hypoxemia. The mechanism of production of hypercapnia is unknown; however, we can postulate a myxedematous lesion involving the muscles of respiration or the central nervous system to produce alveolar hypoventilation.

Three patients had hypoxemia without evidence of lung disease. The most reasonable explanation for hypoxemia in these persons is that they had a disturbance in ventilation perfusion ratios, either mixed venous blood perfusion of underventilated lung areas or overperfusion of normally ventilated lung areas. Some support for this comes from the fact that two of the three failed to come to full values for oxygen saturation after breathing 99.6 per cent oxygen for 10 minutes. The third patient has an arterial saturation of 92.4 per cent while breathing room air. This is just below the normal range. These patients did not develop the "Pickwickian Syndrome" as alveolar hypoventilation was not present.

Several of our patients clinically resembled the

"Pickwickian" patient. One example was F. S. who was somnolent, plethoric and obese. While waiting his turn to be seen in the medical clinic he was found sleeping soundly and was promptly called to our attention as an example of this syndrome. Lung function studies were normal. His somnolence had some other cause than hypoxemia or hypercapnia.

SUMMARY AND CONCLUSIONS

We have studied 28 persons who weighed at least 100 pounds more than their ideal weight. Of this group 13 had normal arterial oxygen saturation. Fifteen had hypoxemia and 10 of the 15 had alveolar hypoventilation as manifested by increased arterial P_{CO_2}. Alveolar hypoventilation was associated with myxedema or lung disease.

Obese persons who had normal arterial oxygen saturation had reduced expiratory reserve volumes, reduced maximal breathing capacities, and reduced maximal flow rates. When these patients lost weight, their lung function returned toward normal.

Some obese persons who did not have lung disease did have arterial hypoxemia without hypercapnia. We believe this is caused by a disturbance in ventilation perfusion ratios in the lungs. However, when alveolar hypoventilation is present (arterial hypoxemia and hypercapnia), one should suspect intrinsic lung disease, a disturbance of the respiratory musculature, or a central nervous system lesion. In our experience obese persons with normal lungs, normal respiratory muscles, and an apparently normal respiratory center did not have alveolar hypoventilation. Most obese persons with pulmonary insufficiency are benefitted by weight loss. Presumably the improvement is caused by reduction in the work of breathing. Obese persons who have lung disease need therapy which is directed toward correcting or alleviating the basic lung disease.

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THE MEASUREMENT OF PULMONARY CAPILLARY BLOOD VOLUME AND PULMONARY MEMBRANE DIFFUSING CAPACITY IN NORMAL SUBJECTS; THE EFFECTS OF EXERCISE AND POSITION [1, 2]

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Carbon monoxide combines with oxyhemoglobin at a measurable rather than an infinitely fast rate (1, 2). This fact is important when carbon monoxide is used to estimate pulmonary diffusing capacity. If the rate at which this gas crosses the membrane between alveoli and pulmonary capillaries is greater than the rate at which it can react with oxyhemoglobin, carbon monoxide tension will rise in these capillaries and slow further diffusion. Therefore, the diffusing capacity of the lungs for carbon monoxide is dependent not only on the thickness and area of the membrane between alveoli and capillaries, but also on the volume of blood in the capillaries and the rate at which this blood can react with carbon monoxide. Roughton and Forster (3) have recently shown that pulmonary capillary blood volume and the true diffusing capacity of the pulmonary membrane can be computed from data on the apparent diffusing capacity of the lung for carbon monoxide (D_{LCO}) at different oxygen tensions and *in vitro* values for the rate of reaction of carbon monoxide with oxyhemoglobin contained in red cells (θ).[6] Such computations have obvious importance in disease states in which the true diffusing capacity of the pulmonary membrane (D_M) and the capillary blood volume (V_C) might vary independently. The present paper presents computations of V_C and D_M in 19 normal subjects and the changes produced in V_C and D_M by exercise and alterations in body position.

METHODS

A. Principle. The diffusion of carbon monoxide from alveolus to capillary is opposed by the resistance of the membrane between alveolus and capillary and by a resistance made up of both the rate at which CO combines with oxyhemoglobin and the volume of blood in the capillaries. The total resistance to diffusion then may be likened to two electrical resistances arranged in series. Thus:

Total resistance = membrane resistance + blood resistance.

The apparent diffusing capacity of the lungs for carbon monoxide (D_{LCO}) is essentially flow/pressure and thus analogous to an electrical conductance; its reciprocal ($1/D_{LCO}$), however, is a resistance and represents the total resistance to diffusion. The true diffusing capacity of the pulmonary membrane (D_M), the reaction rate of carbon monoxide with oxyhemoglobin (θ) and the volume of blood in the capillaries (V_C) are also analogous to electrical conductances; their reciprocals express the resistances due to the membrane and to the blood, respectively. Thus, the equation above may be restated symbolically:

$$1/D_{LCO} = 1/D_M + 1/\theta \times 1/V_C. \quad (1)$$

A rigorous mathematical derivation of this equation has been given by Roughton and Forster (3).

θ decreases with increasing pO_2 in the capillary blood, and its value at pO_2's of greater than about 100 mm. Hg (where there is no appreciable reduced hemoglobin) is known from *in vitro* data (2). D_{LCO} may be measured at several different levels of capillary pO_2, corresponding to several different values of θ. If the reciprocals of

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[3] Part of this work was performed while a Fellow of the Rands Foundation.

[4] Fellow of the Rands Foundation.

[5] This work was performed while a Medical Student Fellow of the United States Public Health Service.

[6] θ includes both the rate of reaction of CO with oxyhemoglobin and the rate of diffusion of CO within the red cell.

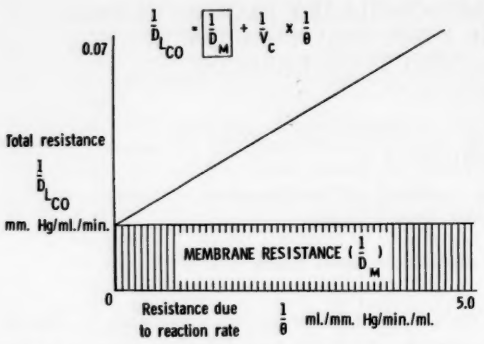

FIG. 1. GRAPHIC DETERMINATION OF MEMBRANE DIFFUSING CAPACITY

A straight line is obtained by plotting the values for $1/D_{LCO}$ against appropriate values for $1/\theta$. The intercept of this line on the vertical axis indicates the resistance due to the pulmonary membrane ($1/D_M$), which is assumed to be constant at any reaction rate.

these measured D_{LCO}'s are then plotted against appropriate values of $1/\theta$ a straight line may be drawn connecting these points (Figure 1). At the point where this line intersects the vertical axis $1/\theta$ is zero and, therefore, the rate of combination of carbon monoxide with oxyhemoglobin is infinitely rapid. Hence, the only resistance to the diffusion of carbon monoxide is that offered by the pulmonary membrane (D_M) and the value of this intercept is equal to $1/D_M$.

If only the triangular area above this intercept is considered (Figure 2) the plotted line is seen to be a graph of blood resistance against reaction rate resistance ($1/\theta$). Since blood resistance is the product of $1/\theta$ and $1/V_C$, the slope of the line must be $1/V_C$, as shown in Figure 2.

B. Experimental procedure. Repeated measurements of D_{LCO} at different oxygen tensions are required to compute V_C and D_M. The single breath technique of Krogh (4) as standardized by Ogilvie, Forster, Blakemore, and Morton (5) is used because it is simple and rapid. Essentially this technique requires that the subject make a maximal inspiration from his residual volume of a mixture containing 0.3 per cent carbon monoxide, 10 per cent helium and varying concentrations of oxygen and nitrogen, hold his breath for 10 seconds, and then make a maximal rapid expiration, from which, after the dead space of both subject and apparatus is cleared, an "alveolar" sample is taken. To vary the oxygen tensions at which these measurements are made and to estimate the tension of carbon monoxide in equilibrium with blood carboxyhemoglobin, we have modified the apparatus described by Ogilvie and associates (5) to the form shown in Figure 3.

The actual conduct of the experiment is as follows: Bag L is filled with a mixture of approximately 0.3 per cent carbon monoxide, 10 per cent helium and 89.7 per cent oxygen.[7] The seated subject breathes 100 per cent oxygen from the demand valve E for two minutes to wash most of the nitrogen from his lungs. Tap F is then turned to J and the subject rebreathes into a 6 liter bag filled with oxygen for four minutes. This constitutes an initial measurement of the carbon monoxide tension in equilibrium with blood carboxyhemoglobin prior to the inspiration of any CO for the experiment (6). The tap is next turned to H and a measurement of diffusing capacity is carried out as described above. The "alveolar" sample is collected in evacuated bag D. At the conclusion of this measurement the tap is turned to E once more and the subject breathes oxygen for an additional two minutes. The tap is then turned to H and the measurement of diffusing capacity repeated. These two measurements constitute a duplicate measurement of D_{LCO} at high oxygen tensions. Next the tap is turned to G and the subject breathes room air for two minutes. The measurement of D_{LCO} is then repeated twice more with a two minute period on room air between the determinations. Since bag L still contains carbon monoxide-helium-oxygen, duplicate determinations of D_{LCO} at alveolar oxygen tensions intermediate between those of preceding and subsequent studies are thus obtained. Bag L is now evacuated and filled with a mixture of 0.3 per cent carbon monoxide, 10 per cent helium, 20.9 per cent oxygen and 68.8 per cent nitrogen. Two more measurements of diffusing capacity are made with two minutes of room air breathing between them—a duplicate determination of D_{LCO} at an oxygen tension close to that of room air. The subject then breathes oxygen for two minutes more and is finally switched to a fresh 6 liter bag (K) filled with oxygen into which he rebreathes for four minutes. This constitutes a final measurement of carbon monoxide tension in equilibrium with blood carboxyhemoglobin. Subsequently the residual volume is determined in duplicate by the closed circuit helium method (7) and venous blood is drawn for determination of hemoglobin by the cyanmethemoglobin method (8).

In experiments to determine the effect of exercise, the subject pedalled for five minutes at a constant rate on a motor-driven bicycle before the first measurement of D_{LCO}, continued to exercise during this determination, during the two minute waiting period and during the duplicate measurement of D_{LCO}. He then rested for five minutes and repeated this procedure at the next level of oxygen tension. This exercise was moderate in degree. In Subject C. D., for example, the O_2 consumption during the fifth minute of exercise was 770 ml. S. T. P. D. The resting residual volume was used in computing D_{LCO}.

In experiments to determine the effects of recumbency, the subject lay down just prior to the initial equilibrium sample and remained in this position throughout the study. Residual volume was determined in the recumbent position. In both the exercise and recumbency studies, a resting seated control study was done on the same day; the control and experiment were done in random order.

[7] Obtained from the Matheson Co., Rutherford, N. J.

C. Gas analysis. Each of the "alveolar" samples to be analyzed is drawn through a dessicating agent by a membrane pump at the rate of 215 ml. per minute and then pushed by the pump through, successively, a paramagnetic oxygen analyzer,[8] an infrared carbon dioxide analyzer,[9] an infrared carbon monoxide analyzer,[10] a carbon dioxide absorber, a hydrator and finally through a katharometer [11] for the measurement of helium. Readings of these instruments are made in all cases while the pump is operating and after the sample has been flowing through this system for three to four minutes. Approximately 540 ml. of gas is required to clear all the analytical apparatus. The carbon monoxide concentration, as read, is corrected for the carbon dioxide present by the use of a curve obtained by passing known mixtures of carbon dioxide in air through the carbon monoxide analyzer.

The helium measurement requires two corrections: One is necessitated by the fact that the katharometer measures the total thermal conductivity of all gases in the sample, rather than helium specifically. The thermal conductivities of oxygen and nitrogen differ; thus, variations in the concentrations of these two gases alter the total thermal conductivity and hence the observed percentage of helium. For this reason the katharometer has two scales designated "helium in oxygen" and "helium in air." Helium concentration of alveolar samples at high oxygen tensions and at oxygen tensions approximating that of room air can be read directly on their respective scales. Samples having oxygen tensions intermediate between these two are read on the "helium in oxygen" scale and corrected to their true helium concentration by a correction curve derived from the analysis of known dilutions of a helium-carbon monoxide-oxygen mixture with air. The second correction is for the carbon dioxide absorbed, since this decreases the volume of the sample and thereby increases the observed percentage of helium. This is accomplished by multiplication of the observed helium percentage by $1 - \frac{CO_2\%}{100}$.

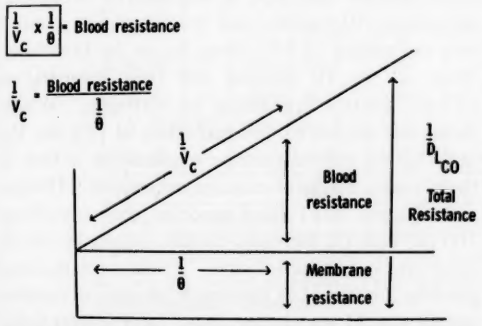

FIG. 2. GRAPHIC DETERMINATION OF CAPILLARY VOLUME

The line itself is a plot of blood resistance against reaction rate resistance per ml. of blood ($1/\theta$); its slope, therefore, is the reciprocal of capillary volume ($1/V_C$).

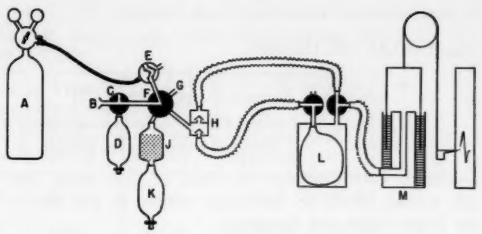

FIG. 3. SCHEMATIC DIAGRAM OF APPARATUS

A is oxygen tank; B is mouthpiece; C is three way tap; D is 6 liter rubber bag; E is demand valve; F is five way tap; G is opening to air; H is flutter valve; J is soda lime canister; K is 6 liter rubber bag; L is 25 liter bag-in-box; M is recording spirometer.

D. Calculations. The initial CO concentration in the alveoli is obtained by multiplying the concentration of CO inspired by the ratio helium expired/helium inspired. From this calculated CO concentration and from the directly measured expired CO concentration is subtracted the concentration of CO in equilibrium with the carboxyhemoglobin of the blood at the capillary pO_2 at which D_{LCO} is measured (9). This correction is of greatest magnitude when the alveolar oxygen tension is high, as indicated by the Haldane relationship (1):

$$\frac{210 \text{ CO tension}}{O_2 \text{ tension}} = \frac{\text{COHb concentration}}{O_2\text{Hb concentration}}.$$

Carboxyhemoglobin is present in significant quantities in the blood of smokers and increases during the experiment in all subjects. During the four minutes of rebreathing into the bag containing oxygen before and after the experiment, the COHb equilibrates with the gas in the lung-bag system (6). From the tensions of CO and O_2 in the bag, the Haldane relation may be solved for per cent COHb if there is little reduced Hb. The rise in per cent COHb between initial and final equilibrations is assumed to occur in equal increments with each determination of D_{LCO}. Thus an interpolated value for mean COHb during each measurement of D_{LCO} is obtained. With this value the Haldane relation can be solved for the CO tension during each measurement of D_{LCO} and this tension (or concentration) may be subtracted from both the initial and final concentrations of CO.

The alveolar volume is the sum of the volume inspired, corrected to S. T. P. D., and the independently measured residual volume. The time of breath holding is measured directly from the respiratory record. D_{LCO}

[8] Model E 2, Beckman Instrument Co., Pasadena, Calif.

[9] Model 16, Liston Becker Division, Beckman Instruments, Stamford, Conn.

[10] Model 15, Liston-Becker Division, Beckman Instruments, Stamford, Conn.

[11] Cambridge Instrument Co., New York, N. Y.

is then calculated from the Krogh equation (4):

$$D_{Lco}\ (\text{ml. CO/mm. Hg/min.}) = \frac{\text{Alveolar vol. (S. T. P. D.)}}{\text{minutes} \times (\text{barometric pressure} - 47)} \times \ln\left[\frac{\text{initial CO conc. in exp. sample}}{\text{final CO conc. in exp. sample}}\right].$$

Duplicate determinations of D_{Lco} at the same oxygen tension which do not agree within 10 per cent of the larger value are discarded.

Values for $1/\theta$ for the alveolar oxygen tension at which each measurement of D_{Lco} is made are obtained from the published graphs of Roughton and Forster (3), using a value of 2.5 for the ratio of permeability of the red cell membrane to the permeability of the red cell interior (λ). These graphs are constructed for blood having a carbon monoxide (or oxygen) capacity of 20 volumes per cent. Corrections for the hemoglobin concentration (Hb) of the subject are made by multiplying the value of $1/\theta$ from the graph by the fraction $\frac{20}{Hb \times 1.34}$, where 1.34 is the oxygen capacity per gram of Hb.

Values for $1/D_{Lco}$ are next plotted against corresponding values for $1/\theta$; the line connecting these points is either drawn by eye, or the best fitting line is computed by the method of least squares. We have used the latter procedure in computing all the data in this study and we have rejected an experiment if the correlation coefficient between $1/D_{Lco}$ and $1/\theta$ is less than 0.95, indicating that more than 10 per cent of the variation in $1/D_{Lco}$ is not explained by changes in $1/\theta$. From the slope and intercept of this line V_C and D_M are obtained as explained above.

RESULTS

In Table I are listed the age, sex, height, weight, body surface area, vital capacity, D_{Lco} while breathing room air, V_C and D_M of 19 normal subjects studied on 23 occasions. In Table II are the correlation coefficients between V_C and D_M and the parameters height, weight, body surface area, vital capacity and D_{Lco} (room air). Except for the correlation coefficient of 0.40 between height and D_M, all these correlation coefficients are significant at the 5 per cent level. In Table III are shown the results of repeated estimates of V_C and D_M over a period of four months in four subjects. We have included in this table a number of studies in which V_C and D_M were computed from four determinations of D_{Lco} at two different oxygen tensions and in which residual volume was assumed to be the same as on some previous study, since such studies might be expected to be more variable than studies performed as described above. The good reproducibility of V_C (coefficient of variation 8.1 per cent) and the wider variability of D_M (coefficient of variation 20.6 per cent) are apparent. Table IV shows the changes produced by moderate exercise in four normal subjects. There is uniformly an increase in V_C and D_M. Table V shows the changes produced by the recumbent position in four normal subjects. There is a uniform increase in V_C, but a decrease in D_M.

DISCUSSION

Accuracy of method

There is no direct method for measuring V_C and D_M. The accuracy of the computed values of V_C and D_M reported here must be evaluated *a priori* from the validity of the assumptions made in these computations. There are three such assumptions: *1*) Varying the alveolar pO_2 does not change V_C and D_M; *2*) D_{Lco} is measurable with accuracy at various pO_2's; *3*) accurate values for θ are available.

Obviously, there is no direct way of proving that alterations in alveolar pO_2 do not change V_C and D_M. Three lines of evidence, however, suggest that such a change does not occur. Çander and Forster (10) have shown that D_{Lco} at an alveolar pO_2 of, for example, 300 mm. Hg is the same whether this pO_2 is attained by breathing oxygen for 10 minutes and then inspiring a mixture containing CO in room air, or by breathing room air for 10 minutes and then inspiring a CO–O_2 mixture containing no nitrogen. While these data do not exclude an effect of pO_2 on V_C and D_M, the only alternative explanation is that if there is any change it must be very rapid. Dripps and Comroe (11) have reported that breathing 100 per cent O_2 increases cardiac output by about 8 per cent in normal subjects. From the data compiled by Forster (12) this much increase in cardiac output should not change D_{Lco} (and inferentially V_C and D_M) appreciably. Lastly, the high correlation between $1/D_{Lco}$ and $1/\theta$ routinely obtained in these experiments suggests that most, if not all, of the change in D_{Lco} with changing pO_2 is due to changes in θ and not to changes in V_C and D_M.

The validity of the second assumption, that D_{Lco} is measurable with accuracy, may be questioned since the various methods for measuring D_{Lco}

TABLE I

PHYSICAL CHARACTERISTICS, VITAL CAPACITY, D_{LCO} (pO_2 c.120), V_c AND D_M OF 19 NORMAL SUBJECTS

Subject	Sex	Age	Height	Weight	Surface area	Vital Capacity	D_{LCO}*	D_M	V_c
		yrs.	inches	pounds	sq. m.	ml. BTPS	ml/mm. Hg/hr in.	ml/mm. Hg/min.	ml.
P.F.	M	23	67.0	163	1.85	4,930	30.0	107.6	65.8
J. J.	M	26	73.0	141	1.86	4,426	29.7	111.6	69.2
E.W.	M	24	75.0	163	2.02	5,770	41.9	200.2	90.9
R.J.	M	27	71.8	170	1.98	5,200	33.9	95.9	70.0
R.U.	M	26	71.5	182	2.04	5,700	37.3	168.5	79.4
D.M.	M	23	71.0	165	1.94	5,210	41.6	110.4	89.8
B.L.	M	32	71.0	155	1.90	4,225	28.4	83.8	65.7
							30.0	87.0	72.3
R.K.	M	21	68.5	158	1.86	5,750	34.9	181.3	68.1
							34.5	145.4	66.4
T.L.	M	31	64.0	127	1.60	3,280	22.8	48.9	65.1
							25.3	82.0	57.3
C.D.	M	22	71.0	167	1.95	5,140	28.9	72.1	67.7
G.L.	F	27	63.0	121	1.56	2,664	23.5	67.8	67.3
M.W.	F	24	62.0	124	1.56	3,605	22.8	88.1	55.3
F.N.	F	34	65.5	156	1.79	3,345	21.6	63.5	50.4
							18.9	81.2	40.7
G.H.	F	29	68.0	135	1.73	3,830	30.0	103.1	74.2
M.T.	F	22	62.5	115	1.52	2,870	23.2	54.3	68.6
C.Y.	F	22	63.0	105	1.46	2,686	18.3	114.3	38.5
Z.K.	F	26	62.5	108	1.48	3,126	21.9	93.5	52.3
V.E.	F	24	62.5	110	1.49	2,970	24.4	58.6	61.7
C.W.	F	33	66.5	130	1.68	4,090	18.8	38.0	59.3
Mean values			66.8	143	1.76	4,149	27.9	98.1	65.0
Standard deviation			4.4	22.2	0.184	1,057	6.8	40.9	12.5

* The following abbreviations are used in all tables: D_{Lco} = apparent diffusing capacity of the lung for carbon monoxide; V_c = capillary blood volume; and D_M = true diffusing capacity of the pulmonary membrane.

give different results. The steady state methods which use either an alveolar sample (13) or the physiological dead space (14) to arrive at a value for alveolar pCO give lower values than the single breath technique (5). The steady state methods are inaccurate unless ventilation/diffusing capacity ratio is the same for all alveoli (9). Ventilation is uneven to some degree in normal subjects (15). Further, the alveolar sample technique faces the practical difficulty of deciding what is the correct alveolar sample and the physiological dead space technique requires skilled technical assistance and is quite sensitive to small analytical errors when used in the resting subject (12). The single breath technique will give a result other than the true D_{Lco} when the ratio diffusing capacity/

TABLE II

RELATION OF PHYSICAL CHARACTERISTICS, VITAL CAPACITY, AND $D_{L_{CO}}$ TO V_c AND D_M

	Correlation Coefficients	
	V_c *	D_M
Height	0.61	0.40
Weight	0.51	0.48
Surface Area	0.61	0.54
Vital Capacity	0.65	0.72
$D_{L_{CO}}$ (pO_2 c. 120mm.Hg.)	0.87	0.74

* For explanation of abbreviations, see Table I.

alveolar volume is not the same for all alveoli (9). It is probable that this ratio is uneven in normal subjects (16). However, the technique is rapid and the gas analysis required is easily done by physical means. In our hands it has been possible to complete duplicate determinations of $D_{L_{CO}}$ at three oxygen tensions within one-half hour.

We have used the single breath technique because of this rapidity and simplicity, because steady state methods have no compelling theoretical advantages over single breath method and because steady state methods would measure the diffusing capacity due to capillaries in which blood flow was very slow or even stopped to a much smaller extent than would a single breath method (14). Thus steady state methods might give a better picture of the functioning capillary bed while a single breath method might give a more accurate picture of anatomical state of this bed. In this connection, Roughton and Forster (3) have compared the values of V_C and D_M obtained by a single

TABLE III

Repeated Determinations of V_c and D_M in the Same Subject

Subject	Date	$D_{L_{CO}}$† ($p\,O_2$ c. 120mm.) ml/mm.Hg/min.	V_c ml.	D_M ml/mm.Hg/min.
B.L.	7/15	29.0	65.7	83.8
	8/27	30.5	72.3	87.0
	10/2	32.8	70.4*	108.6*
	11/7	29.7	66.3*	82.5*
T.L.	7/17	24.2	65.1	48.9
	8/26	25.5	57.3	82.0
	9/25	22.9	52.9*	75.2*
	9/26	23.1	50.9*	67.0*
	10/7	22.4	54.2*	52.0*
R.K.	7/16	35.0	68.1	181.3
	8/28	34.5	66.4	145.4
F.N.	7/23	21.7	50.4	63.5
	8/29	18.9	40.7	81.2
	10/23	17.0	52.5*	33.5*

* Based on 4 measurements of $D_{L_{CO}}$; Residual volume assumed not to vary from previous measurements.

† For explanation of abbreviations, see Table I.

TABLE IV

EFFECTS OF EXERCISE ON V_C AND D_M

Subject	V_C* ml.		D_M ml/mm. Hg/min.	
	Rest	Exercise	Rest	Exercise
T.L.	57.3	62.9	82.0	137.8
B.L.	72.3	85.3	87.0	122.5
C.D.	67.7	84.6	72.1	86.2
C.W.	59.3	70.3	38.0	54.3
Mean	64.1	75.8	69.8	100.2

* For explanation of abbreviations, see Table I.

breath method and by a steady state method and found that V_C and D_M computed from steady state data were, on the average, lower. However, values of V_C and D_M computed by the two methods overlapped and were not vastly different.

The values of θ used in these studies were taken from the published data of Roughton and Forster (3) and the probable accuracy of the measurement of θ has been comprehensively discussed by these authors. In summary, the most important sources of uncertainty in a given value of θ are the value of λ and the value for the pulmonary capillary hematocrit. λ is the ratio of permeability of the red cell membrane to permeability of the red cell interior. The smaller its value, the greater the resistance to diffusion offered by the red cell membrane and the less offered by the alveolar-capillary membrane at the same value of D_{LCO}. Thus, any inaccuracy in the value of λ is reflected in the computation of D_M. The computation of V_C, however, is almost independent of the value of λ. Inaccuracy in the value of the pulmonary capillary hematocrit, on the other hand, affects the computation of V_C, but not that of D_M. There is considerable evidence (17) that the pulmonary capillary hematocrit is less than the venous hematocrit. If this is true, the values for V_C reported here are too low, but the magnitude of such an error is not, at present, known.

To summarize, we feel that our measured values for D_{LCO} and the data for θ that we have used give the correct order of magnitude of the processes they purport to measure, rather than absolute values. More cannot be claimed for the computed values of V_C and D_M. Accordingly, the greatest usefulness of these computations lies in demonstrating *changes* in disease states and under altered physiological conditions.

Comparison with other data

Roughton and Forster (3) have presented data on V_C and D_M in seven normal subjects whose D_{LCO} was measured by a 10 second breath holding technique similar to that used in these studies. Their mean value of 78.7 ml. for V_C does not differ significantly from our mean value of 65.0 ml. for 19 subjects while their mean value for D_M, 57.6 ml. per mm. Hg per minute, is significantly lower than our value of 98.1 ml. per mm. Hg per minute. There are three differences in technique and computation between the two sets of data: *1*) Roughton and Forster computed separate values for D_M using reaction rate data at λ equals 1.5 and λ equals infinity, respectively, and used the average of these two values of D_M. We computed D_M using reaction rate data for λ equals 2.5. Recomputation of our data in the manner of Roughton and Forster makes our average D_M still higher and does not explain the difference. *2*) Roughton and Forster subtracted 5 mm. Hg from the oxygen tension of their alveolar samples to obtain mean capillary oxygen tension and subsequently appropriate values for $1/\theta$. If we made this correction our results for D_M would be lower and closer to those of Roughton and Forster. The difference thus introduced, however, is rather small. For example, in Subject E. W., the subtraction of 5 mm. Hg from the oxygen tension of his alveolar samples in obtaining the values for $1/\theta$ would change D_M from 200 to 188 ml. per mm.

TABLE V

EFFECTS OF BODY POSITION ON V_C AND D_M

Subject	V_C* ml.		D_M ml/mm. Hg/min.	
	Sitting	Recumbent	Sitting	Recumbent
T.L.	57.3	87.1	82.0	60.8
B.L.	72.3	101.2	87.0	78.8
F.N.	40.7	66.4	81.2	46.0
R.K.	66.4	88.8	145.4	123.5
Mean	59.2	85.9	98.9	77.3

* For explanation of abbreviations, see Table I.

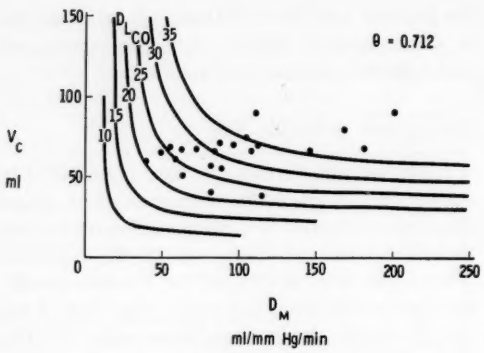

FIG. 4. RELATION OF CAPILLARY VOLUME TO MEMBRANE DIFFUSING CAPACITY

At a fixed value of D_{LCO} and θ, the possible values of V_C and D_M lie on a rectangular hyperbola. A series of such hyperbolae corresponding to the values of D_{LCO} while breathing room air indicated are shown. A value of 0.712 ml. per mm. Hg per ml. blood per minute for θ corresponding to a pO_2 of 120 is used in all cases. Each dot indicates a value for V_C and D_M from Table I.

Hg per minute. *3*) Roughton and Forster analyzed for helium with a mass spectrometer. Our measurement for helium is less specific, since we measured the total thermal conductivity of our samples and interpreted our results as concentration of helium. A systematic error might thus be introduced that would explain the differences between our results and theirs. To explore this point, three "dummy" experiments were done, using a bag containing 2 liters of alveolar air of various oxygen tensions as "lungs." "Alveolar" samples were analyzed and the ratio initial CO concentration in expired sample/final CO concentration in expired sample was computed as described above. There was no significant difference from the expected ratio of 1.0 and thus there is no evidence of a systematic error in our analyses.

Since differences in technique and computation do not provide an adequate explanation, we feel that the most likely reason for the difference in the two sets of data lies in the mathematical relation of V_C and D_M. Rearrangement of Equation 1 to solve for V_C yields

$$V_C = D_M D_{LCO}/\theta(D_M - D_{LCO}). \qquad (2)$$

The possible values of V_C and D_M that would satisfy this equation at fixed values of D_{LCO} and θ fall on a rectangular hyperbola (Figure 4). Practically, this means that when a point falls on the horizontal asymptote, as do most of our points for normal subjects, a small change in V_C will cause a large change in the value of D_M. It is apparent that in most cases small increases in our values for V_C would have brought our values for D_M into the range reported by Roughton and Forster. This same relationship between V_C and D_M, we feel, also accounts for the much wider variability of D_M as compared with V_C which is shown in Table III.

Changes with exercise and position

An increase in D_L with exercise has been observed uniformly by all investigators beginning with Krogh (4). Roughton (18), in 1945, reported that the increase in D_L was due, in part at least, to an increase in V_C on exertion. Our data confirm the increase in V_C observed by Roughton and show that an increase in D_M also occurs. Observations of V_C and D_M on recumbency have not been reported previously, but an increase in D_{LCO} has been shown to occur on changing from the sitting to the recumbent position (5). Our data confirm this observation and indicate that an increase in V_C accounts for this change. An increase in blood in the lung on recumbency, if not in the capillaries, can be inferred from the well-known decrease in vital capacity in the lying position, which is partially reversed by inflating cuffs about the extremities prior to recumbency, a procedure that might be expected to prevent blood from pooling in the lungs (19). The decrease in D_M in our data is not statistically significant because of the great variability in measurements of D_M noted above, and means only that no significant increase occurred.

The observations reported here are of considerable interest in relation to concepts of the physiology of the pulmonary capillary bed. One concept pictures the capillaries as incapable of dilatation and, consequently, either entirely open or completely closed, with only a small fraction of the capillaries of an alveolus open at any one time. This concept is based on the analysis of physical forces in capillaries by Burton (20) and direct observations of capillaries in the cat lung (21), the frog mesentery (22) and the dog lung

(23). This may be called the "open-shut" hypothesis. Alternatively, one might picture some capillaries as containing a small volume of blood and having a folded wall. Under appropriate conditions these capillary walls would unfold, increasing capillary volume with an unchanged surface area ("unfolding" hypothesis). Or, some capillaries containing a small volume of blood might be capable of dilatation, increasing both their volume and their surface area ("dilatation" hypothesis). Our data on exercise, in which both V_C and D_M increase, could be explained by the "open-shut" hypothesis with the opening up of new capillaries, increasing both the volume of blood and the surface area for diffusion. The "open-shut" hypothesis might explain the results of recumbency only if it were postulated that blood went preferentially to the larger capillaries, thereby increasing the volume of blood in the capillaries, but possibly even decreasing the surface area. The "unfolding" hypothesis might explain the increase in V_C without change in D_M on recumbency, but could not explain the increase in both V_C and D_M on exercise. The "dilatation" hypothesis would explain our results with exercise but not those on recumbency. The "open-shut" hypothesis of capillary action in the lung, then, might provide a simple explanation of the changes in V_C and D_M reported here. However, a more complex explanation is not excluded. For example, capillary walls might simply unfold with recumbency, but might be stretched on exercise, increasing their surface area, as Barcroft (24) has suggested, since the functional residual capacity is increased on exercise (25), but is decreased on recumbency (26).

SUMMARY

1) The pulmonary capillary blood volume (V_C) and the true diffusing capacity of the pulmonary membrane (D_M) have been computed on 23 occasions in 19 normal subjects by the method of Roughton and Forster. The average value for V_C was 65.0 ml.; for D_M, 98.1 ml. per mm. Hg per minute.

2) In these 19 subjects V_C and D_M are significantly correlated with height, weight, body surface area, vital capacity and apparent diffusing capacity for CO while breathing room air.

3) In four subjects V_C proved to be stable and reproducible over a period of months while D_M was quite variable.

4) V_C and D_M increased on mild exercise in four subjects.

5) V_C increased while D_M did not vary significantly when four subjects changed from the seated to the recumbent position.

6) The data for V_C and D_M reported here probably give the correct order of magnitude of these quantities rather than their absolute values.

7) The average V_C in our subjects is similar to that reported elsewhere; our average D_M is significantly higher. This may be due to the mathematical relation of V_C to D_M which makes D_M highly variable in normal subjects.

8) The hypothesis that capillaries are either shut or completely open appears to be the simplest explanation of our results both on exercise and during recumbency.

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APPENDIX

The regression equations for the prediction of V_C and D_M from the various parameters measured are as follows:

V_C (ml.) = 1.74 height (inches) − 51.0 ± 9.8

V_C (ml.) = 0.285 weight (pounds) + 24.3 ± 10.7

V_C (ml.) = 41.1 surface area (sq. M.) + 7.0 ± 9.8

V_C (ml.) = 0.00717 vital capacity (ml.) + 35.3 ± 9.4

V_C (ml.) = 1.59 D_{LCO} (ml./mm. Hg/min.) + 20.6 ± 6.1

D_M (ml./mm. Hg/min.) = 3.77 height (inches) − 153.4 ± 37.5

D_M (ml./mm. Hg/min.) = 0.884 weight (pounds) − 27.4 ± 35.9

D_M (ml./mm. Hg/min.) = 120 surface area (sq. M.) − 112.4 ± 34.5

D_M (ml./mm. Hg/min.) = 0.0279 vital capacity (ml.) − 17.5 ± 28.4

D_M (ml./mm. Hg/min.) = 4.46 D_{LCO} (ml./mm. Hg/min.) − 26.4 ± 27.5

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THE "EFFECTIVE" PULMONARY COLLATERAL BLOOD FLOW IN MAN[1]

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Anatomists have long been aware that an extensive pulmonary collateral circulation is a common feature of various types of heart and lung disease (1–5). In recent years, as the result of refinements in methods for the anatomic display of pulmonary vessels, they have been able to gain further insight into the nature and extent of the collateral circulation and to establish that many of the systemic arterial branches join the pulmonary vascular tree proximal to the pulmonary capillary bed (6–11).

Angiocardiography and observations during thoracotomy (12) suggest that these communications may transmit appreciable quantities of blood. Efforts have been made to measure the volume rate of flow through such vessels (13–17). These physiologic studies have been predominantly oriented toward the assessment of the hemodynamic burden which is imposed upon the left heart by the systemic contribution to pulmonary blood flow. In theory, a dye-dilution method could serve this purpose by providing a measure of *total* pulmonary collateral blood flow (18, 19); in practice it has been found more feasible to use the Fick principle, recognizing that this method measures only the "effective" collateral blood flow, *i.e.*, the component of the total collateral blood flow which reaches the alveolar-capillary surface of the lung to participate in gas exchange.

Only few measurements of pulmonary collateral blood flow have been attempted in either animal or man. This paucity stems from: *1*) a lack of suitable animal preparations or human subjects to whom available techniques may be applied; *2*) the inaccessibility of the collateral circulation for direct blood sampling; and *3*) the complexity of the experimental protocols and procedures which are entailed in such studies.

The largest body of data (13) is based on ingenious, but exceedingly indirect, applications of the Fick principle. During recent years, considerable insight has been gained into potential errors which attend the application of the Fick principle to the measurement of blood flow even under *ideal* experimental circumstances (20–22). The indirect applications have exaggerated these sources of error in several major respects: *1*) the use of serial rather than simultaneous sampling of the inter-related blood and gas phases; *2*) the existence of fluctuating levels of anesthesia; and *3*) the administration of 100 per cent oxygen to fix the oxygen content of pulmonary venous blood in a situation where the quantity of dissolved oxygen cannot be accurately predicted (13).

The experiments described in the present report were designed to measure "effective" rather than total collateral blood flow in man. They extend the earlier observations in several directions: *1*) the use of a more direct Fick method; *2*) the avoidance of general anesthesia; and *3*) the application of these methods to a variety of clinical states in which the existence of a pulmonary collateral circulation has been inferred or anatomically demonstrated.

METHODS AND MATERIALS

SUBJECTS

The present report is concerned with 12 subjects who had some anatomic basis for a pulmonary collateral circulation. Their anatomic abnormalities were such as to lend themselves to classification into three groups: *1*) prolonged obstruction of a major pulmonary artery, so that one lung is perfused solely by systemic arterial blood;

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TABLE I

Vital statistics and clinical description of all subjects

Subject	Sex	Age	Surface area $M.^2$	Clubbed digits	Clinical description
			Perfusion of one lung solely by systemic arterial blood		
O. B.	F	34	1.60	0	Status 11 years post ligation of left pulmonary artery
A. B.	M	71	1.65	++	Status 6 months post occlusion of left pulmonary artery by bronchogenic carcinoma
			Perfusion of one lung by both mixed venous and systemic arterial blood		
J. S.	M	42	1.69	++	Bronchogenic carcinoma of right upper lobe
J. Mc.	M	55	1.95	++	Bronchogenic carcinoma of left upper lobe
P. C.	M	54	2.13	0	Bronchogenic carcinoma of right lower lobe
J. C.	M	54	1.71	0	Bronchiectatic and cystic changes in right middle and lower lobes, 10 years after Friedlander's pneumonia
T. C.	M	53	1.93	++	Bronchiectasis of right lower lobe
J. G.	M	27	1.57	++++	Idiopathic clubbing of digits recognized in early childhood
			Perfusion of both lungs solely by systemic arterial blood		
J. W.	F	24	1.55	++++	Tetralogy of Fallot; atresia of pulmonary artery
W. Y.	M	30	1.77	++++	Tetralogy of Fallot; atresia of pulmonary artery
A. K.	F	5	0.50	++++	Tetralogy of Fallot; atresia of pulmonary artery
B. C.	M	13	1.10	+++	Tetralogy of Fallot; atresia of pulmonary artery

2) unilateral intrinsic pulmonary disease, so that one lung is perfused both by mixed venous blood through the pulmonary artery and by systemic arterial blood through the collateral vessels; and *3*) congenital absence of a normal pulmonary artery associated with either atresia of the main pulmonary artery or a truncus communis, so that both lungs are perfused solely by systemic arterial blood.

The members of the three groups are briefly described in Table I. For the sake of convenience, the patient with idiopathic clubbing of the digits is included in Group II.

GROUP I. PERFUSION OF ONE LUNG SOLELY BY SYSTEMIC ARTERIAL BLOOD

This group consists of two subjects in whom blood flow through the left pulmonary artery had been arrested; however, the cause of the obstruction, and its duration, was strikingly different in the two cases.

Subject O. B. has been described in a previous publication from this laboratory (23). In brief, she had undergone surgical ligation of the left pulmonary artery in 1947, during the course of an operation designed to close an infected patent ductus arteriosus. Her clinical course and laboratory studies were reviewed in 1949 to demonstrate the effects on ventilatory and circulatory performance of depriving a lung of its normal perfusion by mixed venous blood. These earlier observations are compared with the pulmonary function studies of 1956 in Table II, and the corresponding bronchospirometry records are reproduced in Figure 1. This table and figure are interpreted as showing that ligation of a major pulmonary artery has only slightly impaired total respiratory function and,

TABLE II

Pulmonary function studies in Subject O. B., measured two and eleven years after ligation of the left pulmonary artery

	Normal	1947	1956
Lung volumes			
Vital capacity*	100	91	99
$\frac{\text{Residual volume}}{\text{Total lung capacity}} \times 100$	25–30	27	28
Maximum breathing capacity*	100	76	100
Alveolar N_2†	<2.5	1.4	2.0
Arterial HbO_2‡			
Rest	96 ± 2	96	96
Exercise	96 ± 2	93	98
Arterial P_{CO_2}§	39 ± 2	39	39
D_{CO}‖			
Rest	14		11
Mild exercise	20		15

* The vital capacity and the maximum breathing capacity are expressed as per cent of predicted, based on previous data from this laboratory.

† Alveolar N_2 = fraction of nitrogen in the lungs after breathing pure oxygen for seven minutes.

‡ Arterial HbO_2 = saturation of hemoglobin in arterial blood, in per cent.

§ Arterial P_{CO_2} = partial pressure of carbon dioxide in arterial blood, in mm. Hg.

‖ D_{CO} = diffusing capacity of the lung for carbon monoxide determined by a steady state method (39) in ml. per minute per mm. Hg.

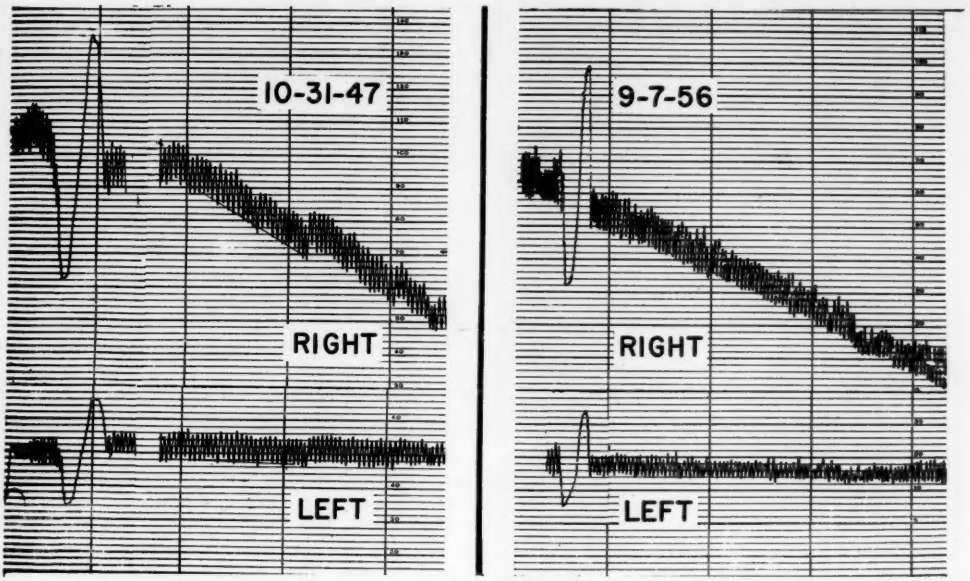

FIG. 1. BRONCHOSPIROMETRIC RECORDS OBTAINED FROM SUBJECT O. B. ON TWO SEPARATE OCCASIONS

Despite an interval of ten years between records, they are strikingly similar. In each instance, oxygen uptake by the left lung is barely perceptible.

moreover, that a stable state has persisted during the intervening 10 years.

In Subject A. B., with a circumscribed mass in the region of the left hilum, contrast substance failed to enter the left pulmonary artery during two separate trials of angiocardiography (Figure 2). At thoracotomy, the left pulmonary artery was found to be occluded by a carcinomatous mass. The duration of the obstruction was estimated to have lasted for no longer than six months.

GROUP II. PERFUSION OF A LUNG BY BOTH MIXED VENOUS AND SYSTEMIC ARTERIAL BLOOD

Each of the six subjects in this group had a clinical state generally associated with a collateral circulation to the lung. As may be seen in Table I, five of the subjects had unilateral pulmonary disease, *i.e.*, bronchiectasis, or bronchogenic carcinoma, and the other had idiopathic clubbing of the fingers and toes. Each of the three patients with bronchogenic carcinoma had a solitary circumscribed lesion, ranging from lemon (J. S.) to orange (P. C.) in size, and affecting only a single lobe; the diagnosis, in each instance, was established by histologic examination of excised lung tissue or lymph node. In the two patients with bronchiectasis, the diagnosis was established by bronchograms; in Patient J. C., an extensive collateral circulation to the affected lung was subsequently visualized at the time of right lower and middle lobectomy.

GROUP III. BOTH LUNGS PERFUSED BY SYSTEMIC ARTERIAL BLOOD

This group consists of four subjects. Their common denominator is a lack of perfusion of the lungs by mixed venous blood, and, perforce, perfusion only by systemic arterial blood.

In Subject J. W. the clinical diagnosis of Tetralogy of Fallot, with atresia of the pulmonary valve, was confirmed at autopsy. In addition, an extensive pulmonary collateral circulation, arising from the internal mammary, pericardiophrenic and intercostal arteries, as well as from the aorta was displayed by injection of plastic material into the thoracic aorta.

In the other three subjects, identical diagnoses were suggested by the clinical picture, and the data obtained during cardiac catheterization and angiocardiography; an alternative diagnosis which could not be excluded, particularly in Subject B. C., was truncus arteriosus. However, from the point of view of the present study, this distinction is unimportant since the method for estimation of collateral blood flow in such subjects is independent of the nature of the congenital malformation as long as both lungs are perfused solely by mixed systemic arterial blood.

PRINCIPLES

The Fick principle was applied to the measurement of collateral blood flow in all three groups; special protocols

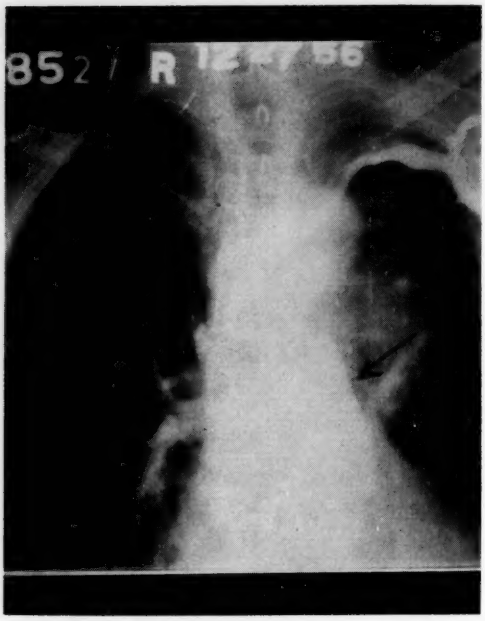

FIG. 2. ANGIOCARDIOGRAM OF PATIENT A. B.

The arrow points to main pulmonary artery, the border of which is sharply outlined by contrast substance. The left pulmonary artery is not opacified.

were required to adapt this concept to the peculiar anatomic circumstances of each group.

GROUP I. PERFUSION OF ONE LUNG SOLELY BY SYSTEMIC ARTERIAL BLOOD

Two inter-related measurements were made: *a*) the volume rate of "effective" collateral blood flow, and *b*) the fraction of total pulmonary blood flow contributed by the "effective" collateral blood flow.

a) The rate of "effective" collateral blood flow through the lung with the occluded pulmonary artery

The method used here is essentially a straightforward application of the Fick principle, entailing: *1*) the use of bronchospirometry to procure gas samples for the calculation of the oxygen uptake and respiratory exchange ratio for each lung separately; *2*) the sampling of peripheral arterial blood for the estimation of the oxygen content of the blood entering the pulmonary capillaries of the affected lung; and *3*) the fixation of the oxygen content of the blood leaving the capillaries of the involved lung by the administration of an appropriate inspired-oxygen mixture. In these broad aspects, the concepts resemble those previously applied to the measurement of unilateral pulmonary blood flow during acute hypoxia (24).

Unfortunately, this application of the Fick principle to a collateral blood flow arising from the systemic circulation is complicated, under the usual circumstances of ambient-air breathing, by the high oxygen content of the afferent blood. The consequences of this situation are illustrated for Patient O. B. in Figure 3 and the actual slopes of oxygen uptake obtained from this patient during ambient-air breathing, on two separate occasions, are illustrated in Figure 1.

The experimental approach used to circumvent this difficulty is illustrated in Figure 4. According to this figure, the lung with the ligated pulmonary artery breathes a slightly enriched oxygen mixture whereas the intact right lung breathes a low-oxygen mixture to induce systemic hypoxemia. By this device, the lung with a systemic arterial blood supply is perfused by hypoxemic blood while being ventilated by an enriched oxygen mixture. The consequences of this arrangement are: *1*) the alveolar-pulmonary capillary diffusion gradient for oxygen is increased, thereby augmenting oxygen uptake; *2*) the pulmonary arteriovenous difference in oxygen content is widened, thereby minimizing the effects of analytic error; and *3*) the hemoglobin in the blood leaving the pulmonary capillaries is fully saturated with oxygen, without unduly increasing the concentration of dissolved oxygen.

In a previous report concerned with *changes* in blood flow, the oxygen content of pulmonary venous blood was equated to the oxygen capacity of systemic arterial blood

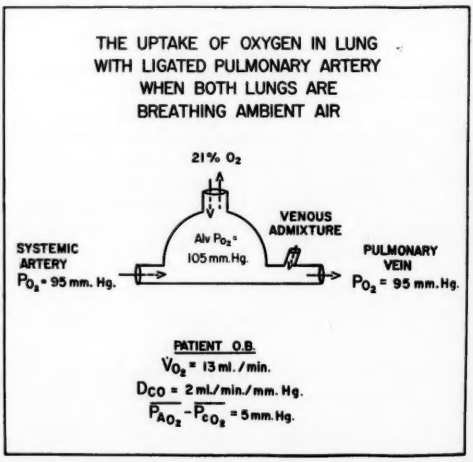

FIG. 3. SCHEMATIC REPRESENTATION OF GAS EXCHANGE IN A LUNG PERFUSED SOLELY BY SYSTEMIC ARTERIAL BLOOD WHILE BREATHING AMBIENT AIR

Since the oxygen tensions (P_{O_2}) in alveolar gas and arterial blood are quite similar, oxygen uptake ($\dot{V}_{O_2}$) is low. From a separate measure of the diffusing capacity of the lung for carbon monoxide (D_{CO}), it may be seen that the mean diffusion gradient for oxygen along the length of the capillary ($\overline{P_{A_{O_2}}} - \overline{P_{c_{O_2}}}$) is abnormally low.

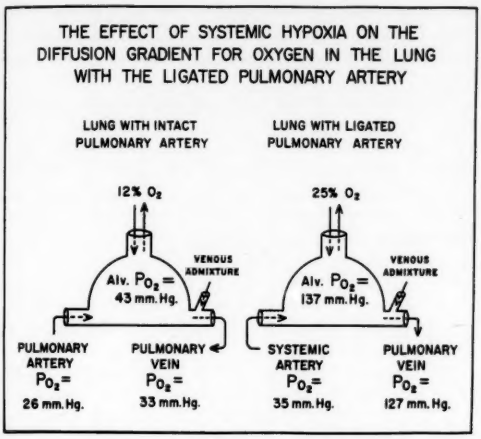
THE EFFECT OF SYSTEMIC HYPOXIA ON THE DIFFUSION GRADIENT FOR OXYGEN IN THE LUNG WITH THE LIGATED PULMONARY ARTERY
LUNG WITH INTACT PULMONARY ARTERY
LUNG WITH LIGATED PULMONARY ARTERY
12% O2
25% O2
VENOUS ADMIXTURE
Alv. PO2 = 43 mm.Hg.
Alv. PO2 = 137 mm.Hg.
PULMONARY ARTERY PO2 = 26 mm.Hg.
PULMONARY VEIN PO2 = 33 mm.Hg.
SYSTEMIC ARTERY PO2 = 35 mm.Hg.
PULMONARY VEIN PO2 = 127 mm.Hg.

FIG. 4. SCHEMATIC REPRESENTATION OF THE EXPERIMENTAL DEVICE USED TO FACILITATE OXYGEN UPTAKE IN THE LUNG PERFUSED SOLELY BY SYSTEMIC ARTERIAL BLOOD

By ventilating the lung with an enriched oxygen mixture (25 per cent O_2) while it is perfused by hypoxemic blood (P_{O_2} equals 35 mm. Hg), suitable pressure gradients are established for the diffusion of oxygen from alveoli to pulmonary capillary blood.

(24); in the present experiments, concerned with *absolute* blood flow, the oxygen content of pulmonary venous blood was assumed to equal the oxygen capacity of arterial blood minus 0.6.[4]

b) The fraction of total pulmonary blood flow contributed by the "effective" collateral blood flow

For this calculation, a measure of the total pulmonary blood flow, *i.e.*, collateral plus pulmonary artery, is required. In this instance, total blood flow cannot be directly obtained by the Fick principle since the two lungs are perfused by blood of different oxygen contents.

Two devices were used to calculate blood flow through the intact pulmonary artery: *1*) a mixing formula, and *2*) a dye-dilution technique.

1) Mixing formula. According to the protocol outlined above for the measurement of "effective" collateral blood flow through one lung, the other *intact* lung is required to breathe a hypoxic mixture. In this circumstance, arteriovenous differences in oxygen content across the intact lung are narrowed and equilibrium between oxygen tensions in alveoli and in the pulmonary capillaries does not occur; consequently, the oxygen content of blood leaving the intact lung cannot be accurately predicted.

For these reasons, a simple mixing formula was applied to the calculation of blood flow through the intact lung; its basis is illustrated in Figure 5. In this calculation the complicating factor of venous admixture is ignored on two grounds: *a*) theoretical, since the effect of venous admixture on arterial blood oxygen content is minimal under the conditions of this experiment, and *b*) practical, since a trial correction of the calculation for venous admixture (26) in Patient O. B. failed to alter appreciably the value for the rate of blood flow through the intact lung.

2) Dye dilution. This method entailed the measurement of blood flow through the intact lung by the Stewart-Hamilton principle (19), following injection of the blue

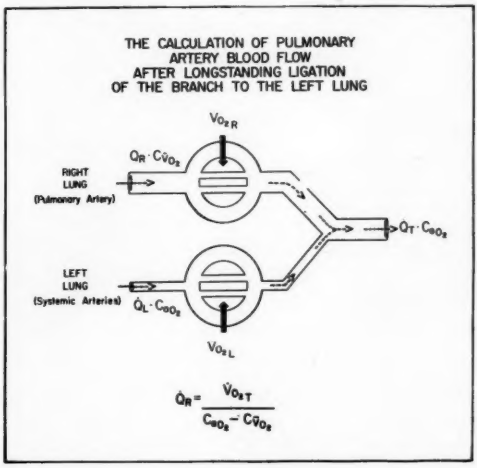
THE CALCULATION OF PULMONARY ARTERY BLOOD FLOW AFTER LONGSTANDING LIGATION OF THE BRANCH TO THE LEFT LUNG
RIGHT LUNG (Pulmonary Artery)
LEFT LUNG (Systemic Arteries)
$\dot{Q}_R = \frac{\dot{V}_{O_2T}}{C_{aO_2} - C\bar{v}_{O_2}}$

FIG. 5. SCHEMATIC REPRESENTATION OF THE BASIS FOR CALCULATING BLOOD FLOW THROUGH THE UNOCCLUDED PULMONARY ARTERY ($\dot{Q}_R$) WHILE THE IPSELATERAL LUNG BREATHES A HYPOXIC MIXTURE

Symbols are discussed in text. The final formulation for $\dot{Q}_R$ is based on the premise that the total rate of oxygen flow from both lungs ($\dot{Q}_T \cdot Ca_{O_2}$) equals ($\dot{Q}_R \cdot C\bar{v}_{O_2} + \dot{V}_{O_{2R}} + \dot{Q}_L \cdot Ca_{O_2} + \dot{V}_{O_{2L}}$). By substituting $\dot{Q}_R + \dot{Q}_L$ equals $\dot{Q}_T$, and $\dot{V}_{O_{2R}} + \dot{V}_{O_{2L}}$ equals $\dot{V}_{O_{2T}}$, the final form of the equation for $\dot{Q}_R$ is easily derived.

[4] The equation of the oxygen content of pulmonary venous blood (Pv_{O_2}) to the oxygen capacity of arterial blood minus 0.6 was empirically derived in the following way. In nine normal subjects, the Fick principle was applied to the measurement of blood flow through a lung breathing a mixture of 25 per cent oxygen and nitrogen (24) by: *1*) measuring ipselateral oxygen uptake, *2*) analyzing mixed venous blood for oxygen content, and *3*) assuming a value for Pv_{O_2} which would yield a normal ratio of right-to-left pulmonary artery blood flow, *i.e.*, 55/45. The average value for Pv_{O_2} obtained under such conditions equaled the oxygen capacity of arterial blood minus 0.6. A similar value was obtained by applying the more elaborate formulation of Cournand, Fritts, Harris and Himmelstein (25) to the same data. It is to be noted that an error in the estimation of Pv_{O_2} has little effect on the calculated collateral blood flows because of the wide pulmonary venous-arterial oxygen differences which characterize these experiments.

dye T-1824 into the main pulmonary artery. On theoretical grounds, this value will exceed that obtained by the mixing formula if: *1*) an appreciable quantity of venous blood entering the intact lung escapes oxygenation by by-passing the alveolar-capillary interface, and *2*) a collateral circulation also perfuses the unoccluded lung. The latter consideration is pertinent only to the subjects of Group II and was circumvented by selection of patients with bronchogenic carcinoma or bronchiectasis affecting only one lung.

GROUP II. PERFUSION OF A LUNG BY BOTH MIXED VENOUS AND SYSTEMIC ARTERIAL BLOOD

In order to apply a similar type of protocol to the measurement of pulmonary collateral blood flow in subjects with intact pulmonary arteries, temporary arrest of blood flow through one major pulmonary artery was effected by inflating a balloon affixed to the tip of an appropriately placed cardiac catheter (27–30). The occlusion of the right pulmonary artery by this device is illustrated in Figure 6. For the sake of visualization during fluoroscopic examination, the balloon was filled with diodrast. Also visible in this figure is the location of the bronchospirometry tube within the tracheobronchial tree.

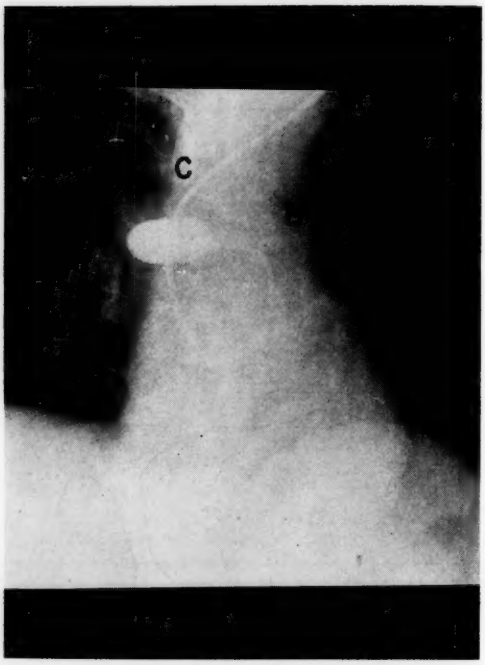

FIG. 6. A BALLOON SITUATED AT THE TIP OF THE CARDIAC CATHETER HAS BEEN INFLATED WITH DIODRAST (A) TO OBSTRUCT THE RIGHT PULMONARY ARTERY

The outline of the bronchospirometry tube is also visible with its tip (B) in the left main bronchus and its special side-arm (C) straddling the carina.

The following criteria were used as indices of complete occlusion of the artery by the inflated balloon: *1*) the cessation of oxygen uptake on the occluded side during ambient air breathing (Figure 7) and its return following deflation of the balloon at the end of the procedure; *2*) the replacement of a normal pulmonary artery pressure pulse, recorded from the lumen distal to the occluding balloon, by an undulating line (Figure 8); this line mirrored only the phasic respiratory variations, and oscillated around a pressure level of only a few mm. Hg greater than atmosphere; and *3*) the withdrawal of fully-oxygenated blood from the distal lumen.

The application of these criteria for complete occlusion of a pulmonary artery in conjunction with others previously proposed for identification of complete separation of the airways to each lung (24) led to the exclusion of six other subjects from this report.

GROUP III. PERFUSION OF BOTH LUNGS SOLELY BY SYSTEMIC ARTERIAL BLOOD

In the case of Tetralogy of Fallot with atresia of the main pulmonary artery, and in truncus communis, both lungs are perfused by systemic arterial blood. In these anomalies, anatomic observations suggest that the collateral vessels probably transport fully-*mixed* arterial blood since the systemic branches generally originate from either the descending aorta or large systemic arteries, or, in the case of truncus communis, from an unusually large vessel. In the face of such anatomic arrangements, a sample of brachial arterial blood may be considered to be identical in oxygen content and capacity to the blood *entering* the pulmonary capillary bed.

On the other hand, the hemoglobin of blood *leaving* the pulmonary capillary bed may be assumed to be fully saturated with oxygen, particularly if a slightly enriched oxygen mixture (25 per cent) is breathed. On grounds discussed in Principles for the calculation of "effective" collateral blood flow through a lung with an occluded pulmonary artery, the oxygen content of blood leaving both lungs is accepted as equal to the oxygen capacity of arterial blood minus 0.6. Also, as in the previous calculations, small errors in $P\bar{v}_{O_2}$ are relatively insignificant because of the wide arteriovenous differences in oxygen content across the pulmonary vascular bed.

CALCULATIONS

GROUP I. PERFUSION OF ONE LUNG SOLELY BY SYSTEMIC ARTERIAL BLOOD

a) The absolute value for "effective" collateral blood flow through the lung with the occluded pulmonary artery (Lung O)

The rate of systemic arterial blood flow through this lung ($\dot{Q}_O$) is equal to the oxygen uptake by this lung per minute ($\dot{V}o_{2_O}$) divided by the corresponding difference in oxygen content between pulmonary venous and systemic

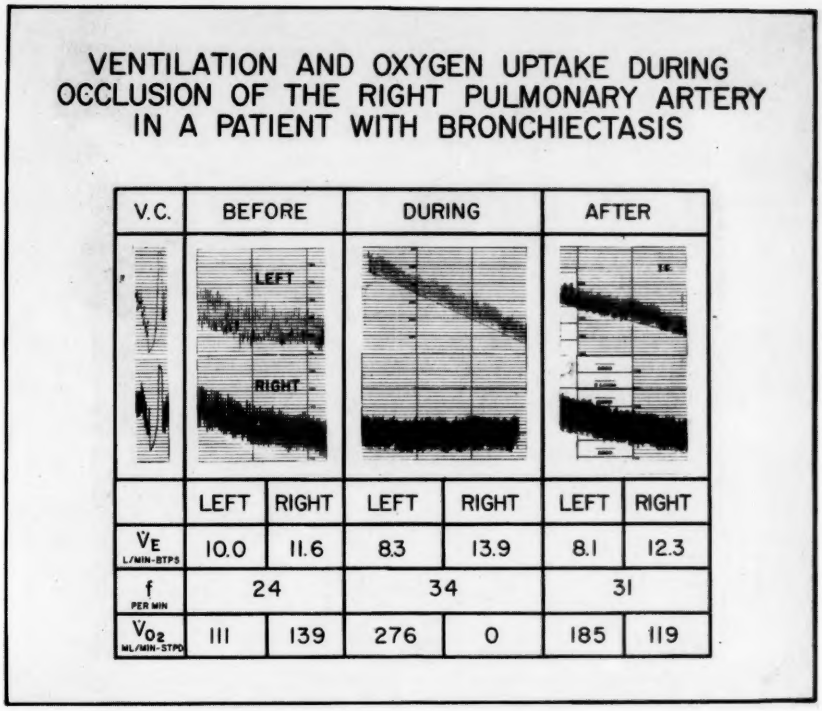

FIG. 7. SPIROGRAMS FROM EACH LUNG SEPARATELY, BEFORE, DURING, AND AFTER OCCLUSION OF A MAIN PULMONARY ARTERY BY INFLATING A BALLOON IN ITS LUMEN

During the period of occlusion, the oxygen uptake on the occluded side becomes imperceptible; following relief of the obstruction, the oxygen uptakes by each side return toward control levels. Abbreviations are as follows: V. C. = vital capacity; $\dot{V}_E$ = minute ventilation; f = respiratory frequency; $\dot{V}_{O_2}$ = oxygen uptake.

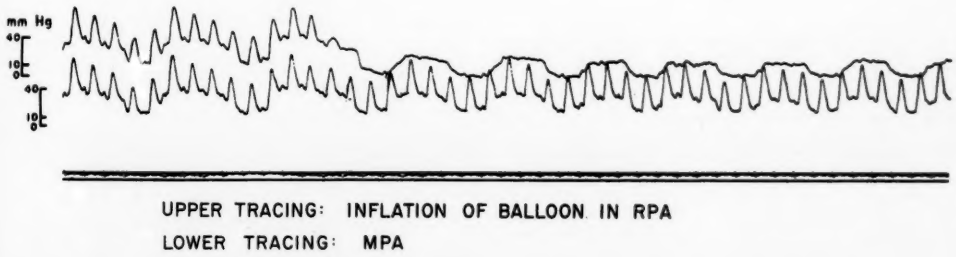

FIG. 8. PRESSURE PULSES RECORDED FROM TWO SITES IN THE PULMONARY ARTERY PRIOR TO, AND FOLLOWING, INFLATION OF AN OCCLUDING BALLOON

As the balloon is inflated, the tracing distal to the occlusion (upper tracing) loses its arterial contour and is replaced by an undulating line which reflects phasic respiratory variations; the pressure pulse in the main pulmonary artery (lower tracing) retains its usual character.

artery blood. For reasons explained under Principles, the calculation was reserved for experiments with systemic hypoxemia and utilized the following formulation:

$$\dot{Q}_O = \frac{\dot{V}o_{2_O}}{(\text{Art. } O_2 \text{ Cap.} - 0.6) - Ca_{O_2}},$$

where,

$\dot{Q}_O$ = the rate of "effective" collateral blood flow through Lung O in liters per minute
$\dot{V}o_{2_O}$ = the oxygen uptake by Lung O, in ml. per minute
Art. O_2 Cap. = the oxygen capacity of systemic arterial blood in ml. per 100 ml.
Ca_{O_2} = the oxygen content of systemic arterial blood in ml. per 100 ml.

b) The fraction of total pulmonary blood flow

The fraction of total pulmonary blood flow contributed by the "effective" collateral blood flow is expressed, in per cent, from the following relationship:

$$\frac{\dot{Q}_O}{\dot{Q}_O + \dot{Q}_P} \times 100,$$

where,

$\dot{Q}_P$ = the rate of blood flow through the unoccluded pulmonary artery.

GROUP II. PERFUSION OF A LUNG BY BOTH MIXED VENOUS AND SYSTEMIC ARTERIAL BLOOD

The calculations used for Group I were applied to data obtained in this group following temporary occlusion of a pulmonary artery.

GROUP III. PERFUSION OF BOTH LUNGS SOLELY BY SYSTEMIC ARTERIAL BLOOD

The rate of "effective" blood flow from the systemic arteries to the gas-exchanging surface of both lungs ($\dot{Q}_T$) was calculated as:

$$\frac{Vo_{2_T}}{(\text{Art. } O_2 \text{ Cap.} - 0.6) - Ca_{O_2}},$$

where,

Vo_{2_T} = the oxygen uptake of both lungs, in ml. per minute.

EXPERIMENTAL PROCEDURE

All subjects were studied in the postabsorptive state, without premedication.

GROUP I. PERMANENT OCCLUSION OF A MAJOR PULMONARY ARTERY

Cardiac catheterization, with placement of the catheter tip in the main pulmonary artery, was done in the usual manner. Atropine sulfate, 0.43 mg., was administered through the catheter lumen. A Carlens tracheobronchial, double-lumen tube was then introduced under local anesthesia. The proper position of the tube was established by fluoroscopy. Following inflation of the occluding bronchospirometric cuffs, the presence of gross leaks was excluded by connecting each lumen to a separate closed circuit containing a six liter spirometer, and then attempting to displace air from one spirometer to the other. After the absence of gross leaks had been established, the brachial artery cannula was introduced. Following a respite from these manipulations, oxygen uptake, minute ventilation and vital capacity were determined for each lung separately, using the closed circuits and the six liter recording spirometers filled with slightly enriched ambient air.

During the control and experimental periods which followed, open circuits were used: Each lung was now part of a circuit which included a tank of oxygen in nitrogen, an anesthesia bag interposed between the reducing valve, and a specially designed, low-resistance, three-way valve[5] and a thirty liter Douglas Bag. Through these open circuits, different inspired oxygen mixtures were delivered to each lung and expired gas was separately collected for sampling and measurement of minute ventilation.

During the control periods, each lung breathed a slightly different mixture of oxygen in nitrogen (ambient air versus 25 per cent oxygen in nitrogen); this difference facilitates the detection of minor leaks between the two tracheobronchial airways from a comparison of the respiratory exchange ratios of the two lungs (24). For reasons explained in Principles, the enriched mixture was supplied to the lung in which a collateral circulation presumably existed. After at least 10 minutes of equilibration on these mixtures, expired gas, systemic arterial and mixed venous blood samples were simultaneously collected for the calculation of blood flow by the Fick principle.

Between control periods, a measured quantity of T-1824 was injected into the main pulmonary artery, through the cardiac catheter, for the estimation of the cardiac output by the Stewart-Hamilton principle (19). Blood samples were collected from the brachial artery, before and after injection, by a constant-drive fractionating device; approximately twenty consecutive samples of blood were gathered over a one minute period. These samples were centrifuged and their plasma analyzed in a Beckman spectrophotometer for their respective concentrations of T-1824. The construction of time-concentration curves and the calculation of cardiac output were then done in the usual manner (19).

After the second control period, and without the patient's knowledge, the low oxygen mixture was substituted for ambient air as the inspired gas mixture to the normal lung; the affected lung continued to receive the *hyperoxic* mixture. After at least fifteen minutes of re-equilibration, the first of two consecutive test periods was terminated by collecting expired gas, arterial and mixed venous blood as in the control periods. The injection of T-1824 and the collection of systemic arterial blood were repeated before the final test period was begun.

[5] Manufactured by Respiration Aids, 255 East 148 Street, New York, N. Y.

GROUP II. PERFUSION OF A LUNG BY BOTH MIXED VENOUS AND SYSTEMIC ARTERIAL BLOOD

In each of the five subjects with unilateral pulmonary lesions (Table I), the pulmonary artery to the *diseased* lung was occluded by inflating a balloon situated on the end of a cardiac catheter. In the sixth subject (J. G.), without a demonstrable pulmonary lesion, the right pulmonary artery was occluded.

The procedure and sequence was that described for Group I, except for the use of a balloon-tipped cardiac catheter to transiently occlude a major pulmonary artery. The catheter contained three lumens; the middle one opened into a latex balloon which was subsequently filled with diodrast, under fluoroscopic control, so as to occlude one pulmonary artery while sparing the main pulmonary artery. Completeness of occlusion was established by briefly substituting the closed circuits described above, for the open circuits. By this device, the cessation of oxygen uptake on the side of occlusion, and the contralateral augmentation, could be directly visualized.

The use of a triple rather than a double lumen catheter afforded several advantages: *1*) Blood pressures in the right ventricle and in the distal pulmonary artery could be monitored as the balloon was inflated; *2*) blood samples could be procured for analysis of blood gas composition proximal and distal to the balloon; and *3*) the dye T-1824 could be injected proximal to the balloon for measurements of blood flow by the Stewart-Hamilton principle.

One threat to the subject during inflation of a balloon in a pulmonary artery is inadvertent encroachment of the balloon on the main pulmonary artery. This possibility was obviated by: *1*) fluoroscopic control of the position of the swelling balloon; *2*) oscilloscopic monitoring of right ventricular blood pressure, particularly during inflation; and *3*) continuous supervision of an electrocardiographic lead on the oscilloscopic screen.

GROUP III. BOTH LUNGS PERFUSED BY SYSTEMIC ARTERIAL BLOOD

All of these subjects had previously undergone diagnostic cardiac catheterization and brachial artery cannulation in the same laboratory. For the calculation of "effective" collateral blood flow, the oxygen uptake was measured in the usual way (31), using an open circuit for the administration of the inspired gas mixture, *i.e.*, 25 per cent oxygen in nitrogen, and a Tissot-type spirometer for the collection of expired gas. An arterial blood sample was drawn during the collection of expired gas.

GENERAL PROCEDURES

In the patients subjected to bronchospirometry and cardiac catheterization, the blood pressures in the pulmonary and brachial arteries were monitored throughout control and test periods. These blood pressures were transduced by Statham strain gauges and were displayed on the oscilloscopic face of a multi-channel apparatus containing high sensitivity carrier amplifiers.

The oxygen content and capacity of whole blood were determined by the Van Slyke-Neill manometric apparatus; the fractions of oxygen in inspired and expired gas were analyzed by the 0.5 ml. Scholander apparatus. All analyses were done in duplicate. From the analyses of inspired and expired gas, the oxygen uptake, the carbon dioxide output, and the respiratory exchange ratios were calculated for each lung, and for both lungs.

RESULTS

The three groups of patients are listed in Table I. For the sake of convenience, the first two groups, *i.e.*, with permanent or temporary occlusion of a pulmonary artery, will be considered together.

Groups I and II. Permanent and temporary occlusion of a pulmonary artery

Control periods. Measurements of gas exchange and blood gas composition in these subjects appear in Table III. Each datum in this table is the average of two consecutive samples, except in Subject J. S. (one control period) and Subject T. C. (one test period). As may be seen in this table, average values for total minute ventilation ($\dot{V}_{E_T}$), for oxygen uptake ($\dot{V}O_{2_T}$), and for carbon dioxide output ($\dot{V}CO_{2_T}$) were similar in the subjects with permanent and with temporary occlusion. Although these values are somewhat high, they are consistent with measurements generally obtained during bronchospirometry (24). Since the increase in total carbon dioxide output paralleled the increase in metabolism, the average respiratory exchange ratio for both lungs was almost normal, averaging 0.81. Arterial blood was well-oxygenated with oxyhemoglobin saturations ranging from 97 to 100 per cent of capacity.

The partition of ventilation and gas exchange differed in the subjects with *temporary* occlusion from those with *permanent* occlusion of a pulmonary artery. Thus, in those with permanent occlusion of a pulmonary artery, both the uptake of oxygen ($\dot{V}O_{2_0}$) and the output of carbon dioxide ($\dot{V}CO_{2_0}$) by the affected lung were abnormally low. Indeed, in Subject A. B. with less than six months of occlusion by carcinoma, gas exchange was negligible. On the other hand, in Subject O. B., with eleven years of occlusion, the oxygen uptake by the affected lung was only four per cent of the total

TABLE III

Ventilation, gas exchange and blood gas composition in subjects with occlusion of one major pulmonary artery

Subject	Side of occluded pulmonary artery*	Experimental state	Gas exchange in lungs: $F_{I_{O_2}}$† Lung O‡	Lung P§	$\dot{V}_E$‖ Lung O	Lung P	Total (L./min.)	$\dot{V}_{O_2}$¶ Lung O	Lung P	Total (ml./min.)	$\dot{V}_{CO_2}$** Lung O	Lung P	Total (ml./min.)	R_E†† Lung O	Lung P	Total	Blood gas composition: Arterial blood O_2 Cont. (ml./100 ml.)	Arterial blood O_2 Sat. (per cent)	Mixed venous blood O_2 Cont. (ml./100 ml)
Permanent occlusion of a major pulmonary artery																			
O. B.	Left	Control	0.25	0.21	5.58	5.16	10.74	13	285	298	54	161	215	4.20	0.57	0.72	19.0	95	15.4
		Hypoxemia	0.25	0.12	8.01	5.93	13.94	61	185	246	57	144	201	0.94	0.78	0.82	12.7	64	10.3
A. B.	Left	Control	0.27	0.21	4.68	9.92	14.60	3	211	214	<1	204	204		0.97	0.95	13.6	100	9.4
		Hypoxemia	0.27	0.12	4.84	9.83	14.67	3	238	241	<1	198	198		0.83	0.82	10.5	82	6.9
Temporary occlusion of a major pulmonary artery																			
J. S.	Right	Control	0.25	0.21	5.16	8.76	13.92	93	190	283	89	160	249	0.96	0.84	0.88	22.4	100	18.4
		Hypoxemia	0.25	0.12	5.58	8.17	13.75	<1	276	277	2	198	200		0.72	0.72	16.9	82	12.8
J. Mc.	Left	Control	0.27	0.21	4.68	5.64	10.32	83	190	273	77	159	236	0.93	0.84	0.86	18.1	95	14.3
		Hypoxemia	0.27	0.12	5.50	8.41	13.91	<1	246	246	12	253	265		1.03	1.08	13.6	72	10.4
P. C.	Right	Control	0.27	0.21	5.74	4.20	9.94	109	156	265	87	140	227	0.80	0.90	0.86	18.4	96	12.9
		Hypoxemia	0.27	0.12	5.59	7.36	12.95	4	226	230	<1	219	220		0.97	0.97	12.9	70	10.6
J. C.	Right	Control	0.27	0.21	6.62	3.79	10.41	160	155	315	126	111	237	0.79	0.72	0.75	19.9	100	15.3
		Hypoxemia	0.27	0.12	6.40	3.74	10.14	22	232	254	43	138	181	1.95	0.60	0.71	13.0	65	9.7
T. C.	Right	Control	0.25	0.21	4.67	3.92	8.59	169	158	327	117	119	236	0.69	0.75	0.72	19.0	96	14.2
		Hypoxemia	0.25	0.12	6.67	6.80	13.47	26	316	342	64	210	274	2.46	0.67	0.80	12.5	64	8.4
J. G.	Right	Control	0.25	0.21	5.14	4.76	9.90	160	185	345	133	148	281	0.83	0.80	0.81	21.9	96	17.6
		Hypoxemia	0.25	0.12	7.51	10.74	18.25	22	347	369	23	324	347	1.00	0.93	0.94	16.0	72	11.8

* In Group II, control periods were completed prior to inflation of the occluding balloon.
† $F_{I_{O_2}}$ = the fraction of oxygen in inspired gas.
‡ Lung O = lung with occluded pulmonary artery.
§ Lung P = lung with patent pulmonary artery.
‖ $\dot{V}_E$ = minute ventilation, in liters per minute.
¶ $\dot{V}_{O_2}$ = oxygen uptake, in ml. per minute.
** $\dot{V}_{CO_2}$ = carbon dioxide output, in ml. per minute.
†† R_E = respiratory exchange ratio.

whereas carbon dioxide production averaged 25 per cent. It is noteworthy that in Subject A. B., the minute ventilation on the affected side ($\dot{V}_{E_O}$) was unduly low. This deficiency was ascribed to injury of the left phrenic nerve during exploratory thoracotomy, one month prior to the study. Although this level of minute ventilation is low, it should presumably suffice to provide an appreciable gas exchange in a lung with an unobstructed pulmonary artery.

In the other subjects, prior to occlusion of a pulmonary artery, the partition of minute ventilation and gas exchange approximated normal values. The lung with the artery to be occluded accounted for 50 per cent of the total ventilation and effected approximately 43 per cent of both the total oxygen uptake and the total carbon dioxide output.

For reasons indicated in Principles, the "effective" collateral blood flow could not be calculated for the control periods.

The average blood flow through the intact pulmonary artery was 6.86 liters per minute when calculated by the mixing formula illustrated in Figure 5. The corresponding value by the Stewart-Hamilton method was 7.23 liters per minute, a difference of 5 per cent.

Test periods. These are designated in Table III as hypoxemia. During these periods, the oxyhemoglobin saturation of systemic arterial blood dropped to an average of 71 per cent, with a range from 64 to 82 per cent. The average total minute ventilation for the eight subjects increased, on the average, by 30 per cent whereas the average oxygen uptake and carbon dioxide output remained virtually unchanged from control, *i.e.*, minus 5 per cent and plus 1 per cent, respectively. The respiratory exchange ratio for the eight subjects changed on the average by 0.4 from control.

Although the total oxygen uptake ($\dot{V}_{O_{2_T}}$) and the total carbon dioxide production remained virtually unchanged during the test periods, the partition between the occluded and nonoccluded sides changed considerably in some subjects. Thus, in Patient O. B., with closure of the left pulmonary artery eleven years previously, there was a fivefold increase in oxygen uptake ($\dot{V}_{O_{2_O}}$) above control values to 61 ml. per minute, whereas carbon dioxide output by the same side ($\dot{V}_{CO_{2_O}}$) remained essentially unchanged. Furthermore, in three of the subjects with temporary occlusion of the right pulmonary artery (J. C., T. C. and J. G.), oxygen uptake on the ipselateral side continued after occlusion but decreased to an average value of 23 ml. per minute. In these subjects, carbon dioxide was also put out by the affected lung ($\dot{V}_{CO_{2_O}}$) at a lower rate, averaging 43 ml. per minute, approximately one-third of control values.

By way of contrast, neither the subject with recent occlusion of the left pulmonary artery by carcinoma (A. B.) nor the three subjects with bronchogenic carcinoma in whom the pulmonary artery was temporarily occluded (J. S., J. Mc. and P. C.), manifested an appreciable gas exchange during the test periods.

The "effective" pulmonary collateral blood flow was calculated for the four subjects with appreciable oxygen uptakes during the hypoxemic periods. As may be seen in Table IV, the largest "effective" collateral blood flow, *i.e.*, 860 ml. per minute, occurred in Subject O. B. with long-standing occlusion of a pulmonary artery. Lesser flows, of 300 to 400 ml. per minute, were measured in the two subjects with bronchiectasis. Intermediate values, *i.e.*, 700 ml. per minute, were calculated for Subject J. G. with idiopathic clubbing of the digits. In Table V are listed the individual values for consecutive periods; these values are shown to be remarkably reproducible. This table also indicates that in none of these subjects did the "effective" pulmonary collateral blood flow exceed 8 per cent of the total pulmonary blood flow.

The blood flow through the unobstructed pulmonary artery increased during hypoxia, averaging 21 per cent above control in the eight subjects. The corresponding increase in total pulmonary artery flow by the Stewart-Hamilton method averaged 29 per cent. Although the direction of changes in flow were similar by both methods, this similarity in averages conceals individual discrepancies; the basis for these differences is not clear.

Considerable changes in pulmonary artery blood pressure occurred during the hypoxemic test periods. Indeed, in one instance (J. G.)

TABLE IV

The "effective" pulmonary collateral and pulmonary artery blood flow in subjects with occlusion of one pulmonary artery

Subject	Experimental state	"Effective" collateral blood flow through Lung O *L./min.*	Pulmonary artery blood flow by mixing formula* *L./min.*	Pulmonary artery blood flow by Dye-Dilution Method† *L./min.*
	Permanent occlusion of a major pulmonary artery			
O. B.	Control		8.26	
	Hypoxemia	0.860	10.43	
A. B.	Control		5.32	5.46
	Hypoxemia	‡	6.62	6.46
	Temporary occlusion of a major pulmonary artery			
J. S.	Control		7.08	7.69
	Hypoxemia	‡	7.16	8.10
J. Mc.	Control		7.18	8.34
	Hypoxemia	‡	7.68	9.76
P. C.	Control		4.81	5.96
	Hypoxemia	‡	9.93	
J. C.	Control		6.85	6.49
	Hypoxemia	0.315	7.70	9.61
T. C.	Control		7.39	7.67
	Hypoxemia	0.382	8.34	12.67
J. G.	Control		8.02	8.99
	Hypoxemia	0.352	8.79	9.22

* Calculated as the total oxygen uptake of both lungs ($\dot{V}_{O_{2T}}$) divided by the difference in oxygen content between systemic and pulmonary arterial blood (see Figure 5).

† Calculated on the basis of the Stewart-Hamilton mean circulation time method.

‡ Oxygen uptake (Table III) was too low for calculation of blood flow by the Fick Principle.

pulmonary artery blood pressure approximated systemic blood pressure. These changes will be analyzed in detail in a subsequent report.

Group III. Both lungs perfused by systemic arterial blood

The results on these subjects are summarized in Table VI. All four subjects were polycythemic. The minute ventilations ($\dot{V}_{ET}$) and oxygen uptakes ($\dot{V}_{O_{2T}}$) were greater than normal in all three subjects but the carbon dioxide outputs were correspondingly increased as reflected in approximately normal values for respiratory exchange ratio (R_{ET}). In this group, with congenital heart disease, the youngest child (A. K.) had a greater "effective" pulmonary collateral blood flow than did the adults in the groups with acquired or induced obstruction of a major pulmonary artery. In Subject W. Y., who was comfortable at rest but who manifested considerable exercise intolerance, the cardiac output at rest was approximately 80 per cent of predicted. During exercise the cardiac output remained virtually unchanged. On the other hand, Subject B. C., who became breathless only during strenuous exertion, manifested a normal increase in cardiac output during mild exercise.

DISCUSSION

Several aspects of the present study warrant further consideration. They will be discussed in the following sequence: *1*) the definition and significance of "effective" pulmonary collateral blood flow; *2*) the effects of systemic hypoxemia on the pulmonary collateral blood flow; *3*) the interpretation of the present results in the light of previous observations by others; and *4*) the contribution of the pulmonary collateral blood

TABLE V

Consecutive measurements of "effective" pulmonary collateral blood flow in four subjects

Subject	"Effective" collateral blood flow, through Lung O $\dot{Q}_O$ *L./min.*	"Effective" collateral plus pulmonary artery blood flow by mixing formula* $\dot{Q}_O+\dot{Q}_P$ *L./min.*	Ratio of "effective" collateral to total pulmonary blood flow† $\frac{\dot{Q}_O}{\dot{Q}_O+\dot{Q}_P}\times 100$ %
O. B.	0.946	11.61	8
	0.773	10.92	7
J. C.	0.411	8.81	5
	0.190	7.25	3
T. C.	0.382	8.72	4
J. G.	0.366	8.72	8
	0.338	9.49	7

* See Table IV for definition of terms.

† Flow through collateral blood vessels, expressed as per cent of total pulmonary artery flow. For this calculation, the collateral blood flow for J. G. to Lung 0 was doubled, since both lungs are presumably equally affected; the other patients, with unilateral pulmonary lesions, required no such correction.

flow to the increment in pulmonary artery pressure elicited by acute hypoxia.

1) The definition and significance of "effective" pulmonary collateral blood flow

The collateral blood supply to the lung originates from intercostal and pericardiophrenic, as well as bronchial, arteries. The use of the Fick principle to measure the blood flow through the pulmonary collateral vessels identifies only that fraction of systemic arterial blood which reaches the alveolar-capillary interface to participate in gas exchange. It does not take into account the volume of blood which by-passes the gas exchanging surface of the lung. Consequently, the Fick principle affords only a minimal estimate of the hemodynamic burden imposed upon the left heart by the collateral blood flow.

2) The effects of systemic hypoxemia on the pulmonary collateral blood flow

Systemic arterial hypoxemia is required for the measurement of "effective" pulmonary collateral blood flow by the Fick principle. Since arterial oxyhemoglobin unsaturation is associated with an increase in both cardiac output and in pulmonary artery pressure (32,20), the effects of these hemodynamic changes on the pulmonary collateral blood flow merit assessment.

The increment in cardiac output should not, *per se*, affect the volume rate of pulmonary collateral blood flow. On the other hand, it is to be expected that an increase in pulmonary artery pressure may, by diminishing the perfusion gradient, decrease the rate of blood flow through the systemic-pulmonary arterial communications (33). However, in experiments with unilateral obstruction of a pulmonary artery, the obstruction spares the zone of communications from the pressor response induced in the proximal pulmonary artery by the systemic hypoxemia. Indeed, the blood pressure in this area tends to be unusually low, favoring an augmentation rather than a diminution in collateral blood flow.

It would be of interest to extrapolate from volume rates of collateral blood flow measured

TABLE VI

"Effective" pulmonary collateral blood flow in four patients with congenital atresia of the main pulmonary artery

Subject	State	$\dot{V}_{E_T}$ *L./min.*	$\dot{V}_{O_{2T}}$ *ml./min.*	R_{E_T}	Arterial blood O_2 Cont. *ml./100 ml.*	Arterial blood O_2 Cap. *ml./100 ml.*	$\dot{Q}_T$* *L./min.*
J. W.	Rest	9.53	222	0.83	14.8	24.1	2.54
	Exercise	33.28	382	0.98	12.6	24.5	3.22
A. K.	Rest	4.03	127	0.80	18.4	28.4	1.35
W. Y.	Rest	8.44	221	0.72	20.2	26.6	3.79
B. C.	Rest	5.32	165	0.80	18.3	22.0	5.00
	Exercise	18.40	494	0.94	16.6	22.5	9.30

* The "effective" pulmonary collateral blood flow, $\dot{Q}_T = \frac{\dot{V}_{O_{2T}}}{(\text{Art. } O_2 \text{ Cap.} - 0.6) - (\text{Art. } O_2 \text{ Cont.})}$

during systemic hypoxemia to corresponding rates of blood flow during ambient-air breathing. Unfortunately, such attempts are complicated by: *1*) uncertainty as to the effects of acute hypoxemia on the collateral blood flow, and *2*) anatomic demonstrations (34) that the collateral blood vessels may not be regarded as passive conduits since they are muscular structures with anatomic potential for altering blood flow, independent of the blood pressure gradients measured at the entrances and exits of these vessels.

3) The interpretation of the present results in the light of observations by others

There is ample anatomic evidence that a variety of chronic pulmonary diseases is associated with large pulmonary collateral circulations (3). Moreover, there is little question that some of these systemic vessels communicate with the precapillary segments of the pulmonary artery (7,11). The present studies in subjects with bronchiectasis and cystic disease of the lungs, by measuring appreciable "effective" collateral blood flows, confirm the presence of precapillary communications in these types of pulmonary disease.

A particularly useful experimental device for stimulating the development of a large pulmonary collateral circulation, has been the ligation of a major pulmonary artery in dogs (8). The luxuriant overgrowth of systemic vessels in the young animals stands in sharp contrast to the meager collateral circulation which develops when the same procedure is performed in adult dogs. It is consequently of considerable interest to note that *children* with congenital atresia of the pulmonary artery may develop larger collateral blood flows than do *adults* with long-standing acquired occlusion of a pulmonary artery.

Physiologic measurements and anatomic observations are far less consistent in the subjects with bronchogenic carcinoma. Thus, although anatomists have repeatedly demonstrated a systemic arterial blood supply to primary carcinomas of the lung (35), the present studies failed to demonstrate an "effective" collateral blood flow to the lung containing the tumor. This discrepancy may be resolved on the ground that the blood supply to the tumor is either exceedingly small or else does not participate in gas exchange, thereby escaping detection by the Fick principle.

Of particular interest with respect to the pathogenesis of clubbed digits is the large "effective" collateral blood flow in the subject with long-standing, idiopathic clubbing of the fingers and toes (35). However, interpretation here is tempered by: *1*) the absence of "effective" collateral blood flow in other subjects with clubbed digits (Table I); *2*) the lack of anatomic basis for an extensive collateral circulation in idiopathic clubbing; and *3*) the inability to define the "ineffective" component of collateral blood flow by the Fick principle.

A final word is in order concerning the hemodynamic significance of the collateral blood flow in patients with acquired pulmonary disease. It has been proposed that the augmented pulmonary venous return effected by the collateral blood flow may strain the left heart (7,36). Two lines of evidence suggest that the left heart strain is not a usual consequence of an augmented pulmonary collateral blood flow: *1*) the diminutive "effective" collateral blood flow recorded above in subjects with intrinsic pulmonary disease, and *2*) the lack of clinical, X-ray, electrocardiographic or postmortem (32, 7) evidence of left ventricular enlargement despite anatomic display of an extensive pulmonary collateral circulation.

4) The contribution of the pulmonary collateral blood flow to the increment in pulmonary artery pressure elicited by acute hypoxia

It has now been well established that acute hypoxemia of moderate degree elicits an increment in pulmonary artery pressure (37,20). This response is exaggerated by decrease in the extent and expansibility of the pulmonary vascular tree (38) and is reflected in the present study by the markedly hypertensive levels proximal to the occlusion. It is noteworthy that the pressor response occurs even though the area of enlarged collateral-pulmonary artery communications has been isolated by the obstructing balloon. Such observations would suggest that the collateral circulation is not an essential contributor to the pressor response.

By way of contrast, hypotensive levels exist distal to the balloon at a time when this area is perfused by hypoxemic blood from the systemic circulation and ventilated by an enriched oxygen mixture. The hypotensive levels in the distal pulmonary artery may stem from: *1*) a decrease in blood volume and flow as inflow of blood from the pulmonary artery is arrested; *2*) a lack of vasoconstriction when the hypoxic stimulus is confined, as in the present experiments, to the precapillary segments of the pulmonary vascular bed; and *3*) a combination of *1* and *2*. Further analysis of this phenomenon will be deferred to a subsequent report.

SUMMARY

1) The "effective" pulmonary collateral blood flow was measured in human subjects, with various types of pulmonary and cardiac abnormalities, by special adaptations of the Fick principle.

2) In support of previous anatomic demonstrations of precapillary communications in the lung, "effective" collateral blood flows were demonstrated in a subject with long-standing ligation of a pulmonary artery and in others with bronchiectasis, with cystic disease of the lung, and with idiopathic clubbing of the digits. However, these blood flows did not exceed 8 per cent of the total pulmonary blood flow. No "effective" collateral blood flow could be measured in subjects with either primary carcinoma of the lung or short-term obstruction of a pulmonary artery.

3) By way of contrast, subjects with atresia of the main pulmonary artery displayed large "effective" pulmonary collateral blood flows, which approximated normal values for cardiac output at rest. During exercise, the "effective" collateral blood flow either remained unchanged or increased.

4) These observations are considered with respect to: *a*) the hemodynamic burden imposed on the left heart by the collateral circulation, and *b*) the contribution of the collateral circulation to the pulmonary hypertension elicited by acute hypoxemia. They emphasize the distinction between "effective" and total pulmonary collateral blood flow.

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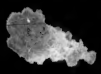
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